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Co-application of mineral fertiliser along with micro-dosed charged biochar's effect on the nutritive statuts and yield of a spring wheat crop

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MYRIAM KAINS

MASTER THESIS PRESENTED FOR THE OBTAINING OF A DEGREE IN
BIOENGINEERING IN ENVIRONMENTAL SCIENCES AND TECHNOLOGIES

ACADEMIC YEAR 2022 - 2023

CO-PROMOTERS: Pr. JEAN-THOMAS CORNELIS & Dr. MARIE DINCHER

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This work was realised in collaboration with the Soil Res3 Lab of the Faculty of Land and Food Systems at the University of British Columbia, Vancouver Campus. The Erasmus$^+$ organisation financed the student mobility.

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Abstract

With a world population recently reaching 8 billion people but a limited surface of arable land, the pressure on cropping systems has never been higher. To continue ensuring global food security, conventional agriculture tends to intensify the input of chemical fertilizers. However, this approach also increases the negative impact these soil amendments have on the environment. Therefore, there is an urgent need to develop new best management practices that fulfill crop's nutritive requirements while reducing the negative impact of the agricultural sector. Given previous findings on biochar-based amendments and co-application, the present study assessed whether co-applying liquid mineral fertilizer along with micro-application rates of charged biochar, would enable a reduction in a crop's normally required fertilizer input without affecting its nutrient status and yield. A 3-month greenhouse trial was set up with 25 acidic coarse-textured soil columns in which spring wheat (*Triticum aestivum* L.) was sown to evaluate the effect of 5 treatments. Charged hardwood biochar was co-applied along with three different rates of fertilizer : 100% (BF100), 66% (BF66), and 33% (BF33) of the recommended application rate. Charged biochar (3 t/ha) (BF0) and 100% of the recommended fertilizer input (F100) were also applied alone and included as treatments. Results showed that treatment BF0 significantly reduced soil NH_4, biomass N_{tot}, and Ca and S mineralomass. However, reducing the amount of fertilizer to 33% (BF33) did not significantly affect any of the analyzed macro and micro-nutrients' soil bioavailability, biomass concentration, or mineralomass compared to the conventional treatment (F100). None of the treatments had a significant effect on crop grain yields. This is hypothetically due to the low alkaline minerals content and micro-application rate of the used biochar. Given that the spatio-temporal experimental limitations may have biased the potential effects of biochar, further long-term and field investigations are needed to evaluate the true potential of this new best management practice, possibly while testing higher, yet still realistic, charged biochar application rates.

Keywords : charged biochar, mineral fertilizer, co-application, best managment practice, yield, nutritive status, spring wheat, soil columns, acidic soil, British Columbia

Résumé

Avec une population mondiale qui a récemment atteint les 8 milliards mais une surface de terres arables limitée, la pression sur les systèmes de culture n'a jamais été aussi élevée. Afin de continuer à assurer la sécurité alimentaire mondiale, l'agriculture conventionnelle a tendance à intensifier l'apport en engrais chimiques. Cependant, cette approche augmente également l'impact négatif qu'ont ses amendements de sol sur l'environnement. Par conséquent, il est urgent de développer de nouvelles pratiques de gestion qui répondent aux besoins nutritifs des cultures, tout en réduisant l'impact négatif du secteur agricole. Compte tenu des résultats antérieurs sur les amendements à base de biochar et la co-application, la présente étude a évalué si la co-application d'engrais minéraux liquides associée à des taux de micro-application de biochar chargé, permettrait de réduire l'apport en fertilisant normalement requis par une culture, sans affecter le statut nutritif et le rendement de celle-ci. 25 colonnes de sol acide et sableux dans lesquelles a été semé du blé de printemps (*Triticum aestivum* L.), ont fait l'objet d'un expérience en serre durant 3 mois afin d'évaluer l'effet de 5 traitements. Du biochar de bois dur chargé (3 t/ha) a été co-appliqué avec du fertilisant à trois taux d'applications différents : 100% (BF100), 66% (BF66) et 33% (BF33) du taux d'application recommandé. Du biochar chargé (3 t/ha) (BF0) et 100% de l'apport recommandé en fertilisant (F100) ont également été appliqués seuls et inclus parmi les traitements testés. Les résultats ont montré que le traitement BF0 a réduit de manière significative les concentrations de NH_4 dans le sol, la biomasse N_{tot} et la minéralomasse de Ca et de S. Cependant, réduire la quantité de fertilisant à 33% (BF33) n'a pas affecté la biodisponibilité des macro et micro-nutriments dans le sol, la concentration de biomasse ni la minéralomasse de manière significative par rapport au traitement conventionnel (F100). Aucun des traitements n'a eu d'effet significatif sur les rendements des cultures. Cela s'explique hypothétiquement par la faible teneur en minéraux alcalins et le taux de micro-application du biochar utilisé. Étant donné que les contraintes spatio-temporelles de l'expériences ont pu biaiser les effets potentiels du biochar, il est nécessaire de mener d'autres essais de terrains à long terme afin d'évaluer le véritable potentiel de cette nouvelle pratique de gestion, éventuellement en testant des taux d'application de biochar chargé plus élevés, mais toujours réalistes.

Mots clés : biochar chargé, engrais minéral, co-application, pratique de gestion, rendement, statut nutritif, blé de printemps, colonnes de sol, sol acide, Colombie-Britannique

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Abbreviations	
BC	British Columbia
BMP	Best management practice
CDC	Critical dilution curve
CEC	Cation exchange capacity
CO_2e	carbon dioxide equivalence
GHG	Greenhouse gas
HI	harvest index
MOP	Muriate of potash
NNI	Nitrogen nutrition index
SDS	Sawdust and shavings
SEM	Scanning electron microscope
SOP	Sulfate of potash

I Introduction

1 Contextualization

Global food security challenge

With a world population of over 8 billion people, the pressure to ensure global food security has never been higher. But with the surface of arable land being limited, conventional agriculture tends to intensify fertilizing inputs to support the ever-growing pressure on cropping systems. Consequently, the adverse effects of this intensification keep increasing, making cropping conditions on earth even more difficult (Nobile et al., 2022). In fact, the agricultural industry was responsible for 10.5 Gt CO_2e in 2020, 70% of which emitted within the farm gate (*World Food and Agriculture – Statistical Yearbook 2022* 2022). Adding the fact that 21% of these emissions were under the form of N_2O, a greenhouse gas (GHG) with a current atmospheric lifetime of 116 ± 9 years (Tian et al., 2020) that has 298 more global warming potential than CO_2 (IPCC, 2014), ways of reducing farmland GHG emissions need to be found urgently if we want to stop affecting Earth's climate negatively (Janzen et al., 2003;Ministry of Environment and Climate Change Strategy, 2022). Since agriculture is one of the most impacting sectors on climate change, but also one of the most sensitive to its effects (Crippa et al., 2021), the challenge lays in ensuring productive, resistant, and resilient cropping systems by using new farming practices that minimize GHG while continuing to ensure global food security.

Agricultural landscape in British Columbia

Crop and animal production account for about 3.6% of the Canadian province of British Columbia (BC) its total emissions as indicated by the BC GHG Inventory reports. As all other farmlands, a major part of these emissions is due to enteric fermentation, manure management, and N_2O emissions from direct & indirect sources. 2021's study on agricultural GHG prepared by the Sustainable Agricultural Landscapes Lab (Smukler, Borden, et al., 2021) showed that within these 3.6%, N_2O emissions, referred to as Agricultural Soils on figure 1, contribute to 22.3% of total agricultural emissions; highlighting the important impact fertilizer have on the environment.

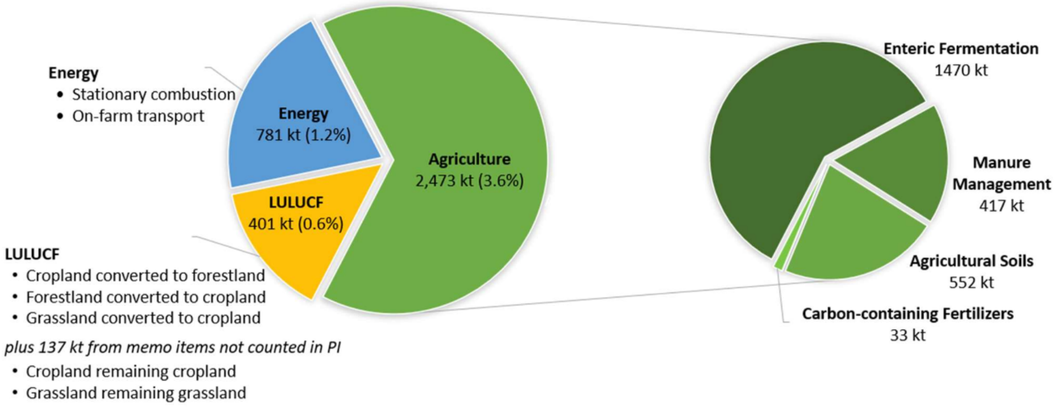


Figure 1: BC's agricultural sector's emissions, total accounting for 5.4% of province's total CO_2e emissions in 2018 (Source : Smukler, Borden, et al., 2021)

Within BC's agricultural regions (figure 2), total field crops and hay account for 519 853ha, 23% of which are allocated to wheat crops (figure 3) (Statistics Canada, 2023), also exposing this part of the world to fertilizer-related problems (Smukler, DeLisa, et al., 2015).

Figure 2: BC's agricultural regions and the Agricultural Land Reserve in darker shades (Source : Dobb and Hackett, 2021)

Within these 23%, spring wheat crops represent 84.3% of BC's total wheat production area (Statistics Canada, 2023) while 1 ha of spring wheat emits 943 kg CO2e, making it the crop with the second largest emission rate (Gan, Liang, Wang, et al., 2011).

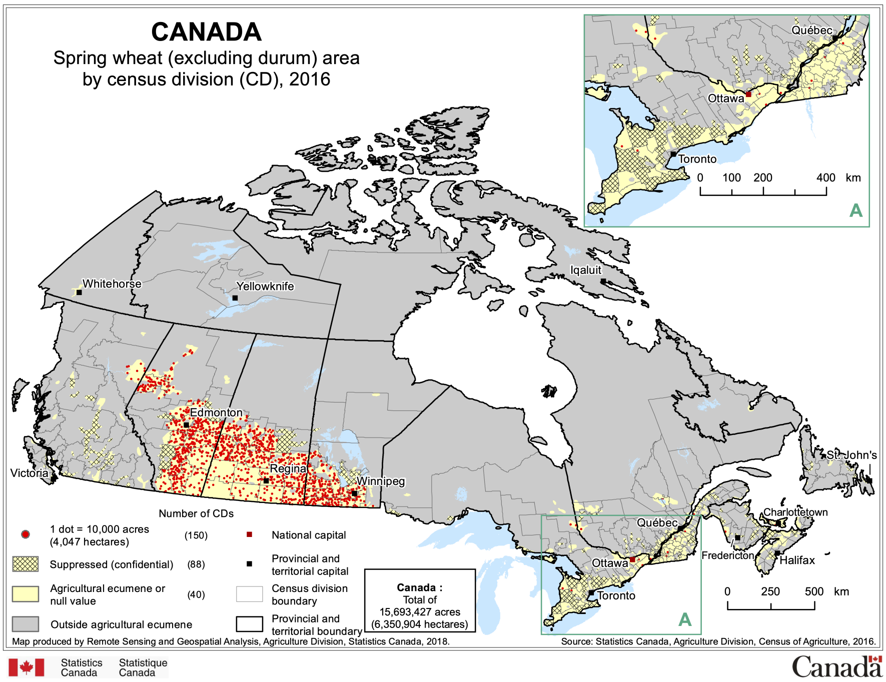


Figure 3: Canada's spring wheat (excluding durum) area by census division for 2016, representing 0.56% of the provincial territory of BC (Source : Statistics Canada, 2023)

2 Spring wheat nutriment requirements

One of the advantages of growing wheat, is that it can be grown on almost any soil. Only, if the aim is to obtain optimal yields, the water supply should not be restrictive, the soil structure needs to present a porous subsoil to allow plants to develop deep roots, soil pH should be slightly acid to neutral and of course, soil fertility should be optimal. The latter plays a key role in establishing if a soil is capable or not of answering the nutrient requirement of a crop. To determine whether this is the case, when growing a crop, the amounts of nutrients required can be derived from soil testing and the nutrient removal through grains and straw (Roy, 2006). Indeed, the different nutrient pools of a cropping system need to be kept in the equilibrium presented in the following equation:

$$restitution + inputs = fixation + outputs + losses + fixation$$

Uptake of nutrients from the soil solution

Available nutrient contained in the soil solution are free to move by mass flow or diffusion or up and down the soil profile with water movement. Plants will take up the major portion of nutrients in this soil solution by using root hairs, i.e. extensions of the epidermal root cells, that vastly expand the root surface area. As the main roots grow, new root hairs are formed allowing a continuous exploration of the soil volume to access available nutrients. Nutrient uptake is therefore affected by root activity and growth (Roy, 2006).

Figure 4 illustrates the nutrient uptake processes in the vicinity of a root hair. Firstly, the nutrient ion enters the apoplast by free flow (e.i. water movement against the nutrient concentration gradient), and thus passing the cell wall tissue of the root hairs passively. Secondly, cations and anions are taken up from the apoplast to the cytoplast by ionophores in exchange for H^+ and bicarbonate ions respectively, this time crossing the cell membrane actively(Roy, 2006).

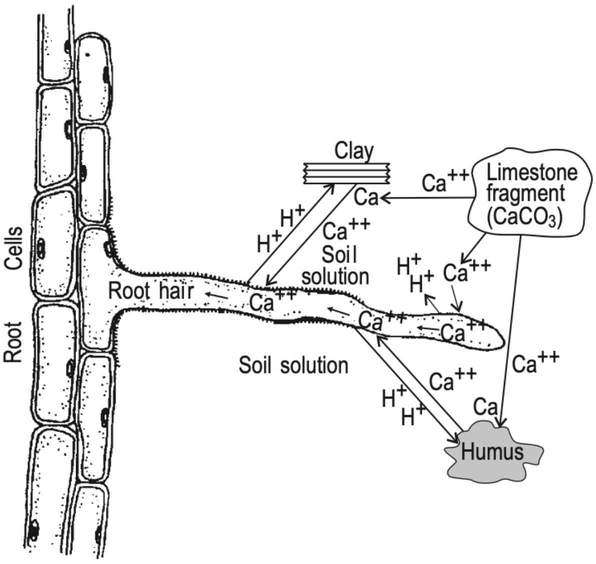


Figure 4: Uptake of nutrients from the soil by a root hair, using Ca as an example (Source: (Roy, 2006)

The fact that nutrient uptake is simultaneously an active and passive process, explains some of its peculiarities. Indeed, plants not only accumulate nutrients against a concentration gradient, but they are also able to select from the nutrients at the root surface according to their requirements (preferential uptake). In addition, owing to their selection capacity, they can exclude unwanted or even toxic substances, but this exclusion capacity is limited. After uptake into the cytoplast, the nutrients are transported to the next cells and finally arrive at the xylem, which is the tissue through which water and dissolved minerals move upward from the roots to the stem and leaves where they are used for photosynthesis and other processes (Roy, 2006).

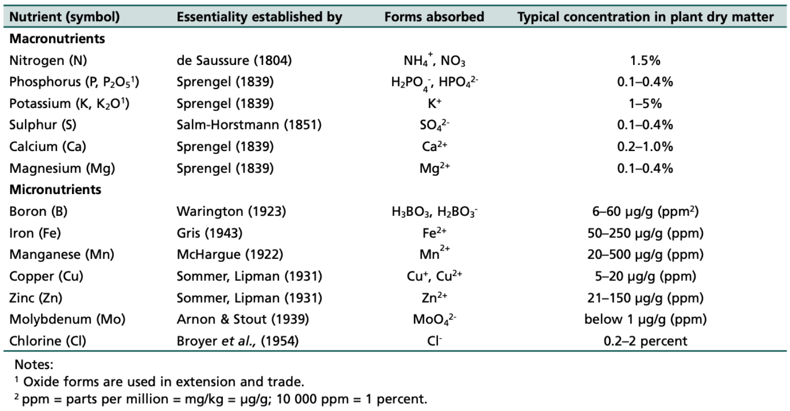
Essential vs. beneficial nutrients

According to Arnon and Stout (1939), there are only 16 elements answering the following criteria and that can therefore be considered as *essential*:

- A deficiency of an essential nutrient makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle.
- This deficiency is specific to the given element and can only be prevented or corrected by providing this element.
- The element's direct involvement in the plant's nutrition is distinct from its potential role in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium.

Out of these 16 elements, three elements make up 95 % of plant biomass: C and O obtained from the gas CO_2, and H obtained from H_2O. They are thus required in large quantities to insure the production of plant constituents such as cellulose or starch. The remaining 5 % are made up of 13 elements taken up in inorganic forms and are therefore called mineral nutrients. According to plant requirements, they can be divided into the two distinct groups of macro- and micro-nutrients even if, physiologically, all of them are equally important. For example, the relative contents of N and Mo in plants is in the ratio of 10 000:1 even though both elements play a key role in biochemical photosynthetic processes, since N is a vital component of chlorophyll and Mo is an essential component of nitrate reductase, the enzyme responsible for converting NO_3^- into NO_2^- (Roy, 2006).

The specific chemical forms in which these 13 mineral elements are taken up by plants and their typical concentration are presented in figure 5.



Nutrient (symbol)	Essentiality established by	Forms absorbed	Typical concentration in plant dry matter
Macronutrients			
Nitrogen (N)	de Saussure (1804)	NH_4^+, NO_3	1.5%
Phosphorus (P, P_2O_5[1])	Sprengel (1839)	$H_2PO_4^-$, HPO_4^{2-}	0.1–0.4%
Potassium (K, K_2O[1])	Sprengel (1839)	K^+	1–5%
Sulphur (S)	Salm-Horstmann (1851)	SO_4^{2-}	0.1–0.4%
Calcium (Ca)	Sprengel (1839)	Ca^{2+}	0.2–1.0%
Magnesium (Mg)	Sprengel (1839)	Mg^{2+}	0.1–0.4%
Micronutrients			
Boron (B)	Warington (1923)	H_3BO_3, $H_2BO_3^-$	6–60 µg/g (ppm[2])
Iron (Fe)	Gris (1943)	Fe^{2+}	50–250 µg/g (ppm)
Manganese (Mn)	McHargue (1922)	Mn^{2+}	20–500 µg/g (ppm)
Copper (Cu)	Sommer, Lipman (1931)	Cu^+, Cu^{2+}	5–20 µg/g (ppm)
Zinc (Zn)	Sommer, Lipman (1931)	Zn^{2+}	21–150 µg/g (ppm)
Molybdenum (Mo)	Arnon & Stout (1939)	MoO_4^{2-}	below 1 µg/g (ppm)
Chlorine (Cl)	Broyer *et al.*, (1954)	Cl^-	0.2–2 percent

Notes:
[1] Oxide forms are used in extension and trade.
[2] ppm = parts per million = mg/kg = µg/g; 10 000 ppm = 1 percent.

Figure 5: Essential plant nutrients, forms taken up and their typical concentration in plants (Source: Roy, 2006)

Besides these 16 essential elements, other elements like Si (Nowakowski and Nowakowska, 1997) or Na (Ashraf and Khanum, 1997) can enhance plant resistance to abiotic stresses like drought, metal toxicity, salinity or improve essential elements availability and are therefore considered as beneficial elements. Without being vital to the crop, they can still improve the growth of a spring wheat crop and may require external addition.

Conventional fertilizers

However, when the concentration of a given essential element is below the critical concentration, i.e. the lowest plant concentration needed to reach maximum crop growth and yield (Fontana et al., 2021), an effective crop nutrition program needs to be dressed to answers crop needs. Even though there are diverse types of materials that can serve as sources for plant nutrition; natural, synthetic, recycled wastes, biological; the majority of nutrient restitution comes from synthetic mineral fertilizers (Roy, 2006).

According to the FAO Roy, 2006, any "mined, refined, or manufactured product containing one or more essential plant nutrients in available or potentially available forms and in commercially valuable amounts without carrying any harmful substance above permissible limits" can be defined as a fertilizer. Furthermore, a fertilizer will be qualified as single if it only contains one of the three major nutrients (N, P or K) and as multinutrient if it contains at least two macro- or micronutrients. In many situations, a suitable multinutrient fertilizer can be selected for the basal dressing followed by a straight fertilizer for top-dressing, to avoid having to buy a separate fertilizer for each nutrient to be applied (Roy, 2006).

Considering the different nutrient ratio combinations, a large number of standard-type NPK fertilizers can be found. Their nutrient concentrations are indicated as percentage of N + P_2O_5 + K_2O, the individual nutrient concentrations ranging from about 5 % to more than 20 %. While a different fertilizer for every crop and field may appeal to sophisticated farmers, the majority of growers use a limited number of standard types. Most NPK types are produced by the acid decomposition of phosphate rock with incorporation of ammonia, thus producing an NP fertilizer to which a K salt, usually MOP or SOP, is added. These can be solid or liquid fertilizers (Roy, 2006).

Liquid fertilizers' nutrient ratios vary in a wide range from 5:8:15 up to 25:6:20 and offer certain advantages. Technically, they enable a more accurate and convenient application of fertilizers on large farms. Indeed, farmers can use the same spraying machines they use for crop protection without the necessity of transporting fertilizer bags—instead, they simply rely on pumping mechanisms. From a chemical standpoint, when applied to a crop, liquid mixed fertilizers sink into the soil and become part of the soil solution, therefore directly and easily available for the plants to absorb through their roots system (Roy, 2006).

Best management practices

Like all manufactured products, the production and use of chemical fertilizers doesn't come without side effects and environmental impact. This is a well know problem that has been the subject of multiple studies (Gan, Liang, Hamel, et al., 2011; Janzen, H. H. et al., 1998; Janzen et al., 2003; Wu et al., 2021, Xu and Lan, 2017) and to which several usage policies, also called best management practices (BMP), attempt to respond.

According to the government of British Columbia [1], the first step towards better nutrient management is analyzing soils samples. This allows farmers to calculate what additional amount of fertilizer they should apply in order to obtain the desired yields knowing what the soil is already capable of providing to the crops.

Another BMP to improve fertilizer use efficiency is the *4R nutrient management strategy.* This consists in applying the right type of nutrient sources in the right amount, in the right place and at the right time. More specifically, this involves using nitrification or urease inhibitors, adjusting application rates based on soil testing, N balance, type of crops and stage of crop development, splitting fertilizer application for different times of the growing season, or practices like subsurface application through banding and/or injection (Smukler, Borden, et al., 2021). According to Smukler, Borden, et al. (2021), considering an actual adoption rate of 60% for all of BC's crops combined, this BMP could save 6.74 kt CO_2e year^{-1}.

However, it is important to realize that the reduction in NO_2 emissions could be offset by the increase of fossil fuel consumption due to split applications. Together with the fact that the usage of slow-release fertilizers or nitrification-inhibitors is not always profitable for producers since it doesn't necessarily include an increase in yield and can include additional costs, explaining why this strategy is not always cost effective. Besides, its applicability is practically limited to certain type of crops and doesn't concern agricultural practices like organic farming who will not be able to use slow-release fertilizers or nitrification-inhibitors (Abedin, 2018; Smukler, Borden, et al., 2021).

3 Biochar-based amendment practices

It has become clear that innovative farm managing practices need to be developed if we want to limit the negative impact agriculture has on the environment. Whitin this vision, biochar has shown great potential (IPCC, 2014).

Biochar

Biochar is produced by pyrolysis of biomass, a anaerobic degradation process at temperatures ranging from 200°C to 800°C. Pyrolysis can be separated into two major techniques, slow and fast, referring to the speed at which the biomass is altered and defining the share of the different co-products of this process (Woolf et al., 2010). Therefore, biochar with a very wide range of properties (e.g., bulk density, specific surface, CEC, pH, mineral composition) can be produced depending on pyrolysis conditions and the origin of the raw material (e.g., wood, food waist, animal manure) (Lévesque, Oelbermann, and Ziadi, 2022). This carbon rich material, chemically identical to charcoal, can only be distinguishes from the latter by its applications. As shown in figure 6, these can be organized into 3 main *pillars*: carbon storage, bioenergy, and soil amendment. By pyrolyzing biomass, its immediate decay is avoided, storing photosynthetically fixed carbon in a more stable form, slowing the flow of CO_2 and other GHG like NO_2 and CH_4 returning to the atmosphere. In addition, three main bio-energy products derive from the pyrolysis process: bio-oil, syngas, and process heat. These can be used to produce energy and offset fossil carbon emissions (Woolf et al., 2010).

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[1] www2.gov.bc.ca, March 2023

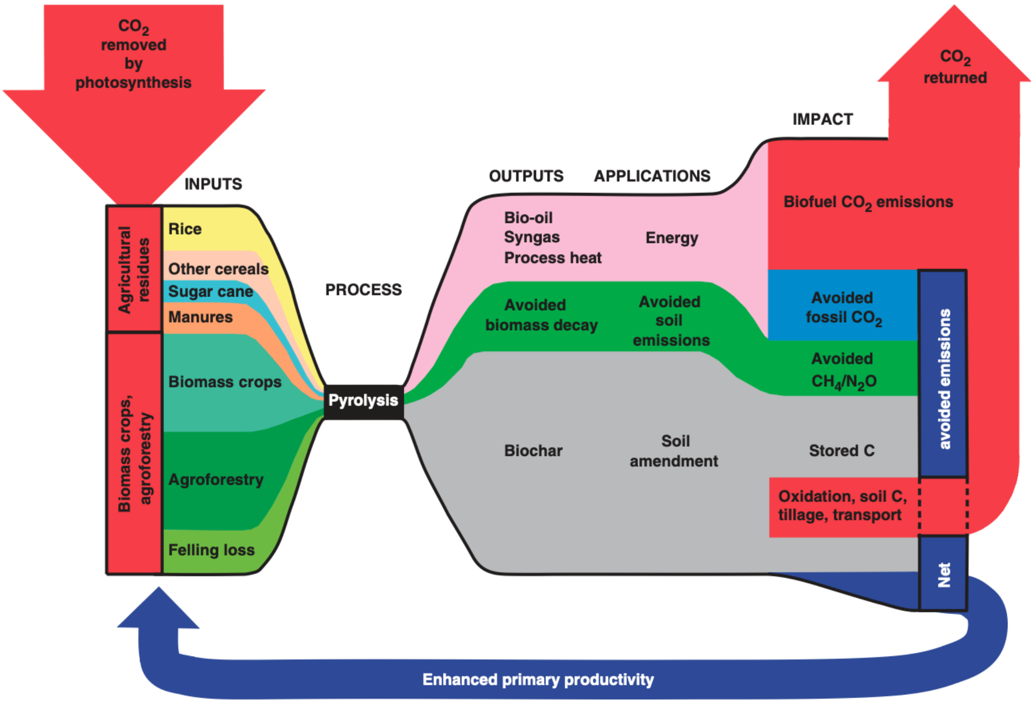

Figure 6: Pyrolyzed organic mater's conceptual cycle (Source: Woolf et al., 2010)

Biochar as a soil amendment

Biochar as a soil amendment is the third and *application pillar* of main interest in this study. Numerous publications have studied the effect of biochar on crop yields and soil fertility, highlighting how these effects can vary with the type of soil considered (Biederman and Harpole, 2013; Ding et al., 2016). Jeffery et al. (2017) showed how applying biochar in acidic and degraded tropical soil can increase crop yields by 25% due to its liming and fertilizing effect, in contrast with its average non-significant effect on temperate soils. Indeed, the latter often have moderate pH values, higher fertility and generally receive higher amounts of fertilizer inputs, leaving less room for improvement through biochar application. Considering the diversity of soils and the number of factors influencing biochar's physical and chemical properties, this explains why the impact of biochar on soil and plant productivity remains uncertain and is very site specific (Lévesque, Oelbermann, and Ziadi, 2022; Nobile et al., 2022).

Charged biochar

While the solid material obtained from pyrolysis is referred to as *pristine* biochar, further research introduced the concept of *charging* biochar. This is a process where pristine biochar gets "loaded" with nutrients by mixing it with animal manure or by co-composting it with plant and animal residues prior to its applications to soils, increasing its beneficial effects on soil fertility (Zwart, 2020). This increase in biochar's positive effect, was explained by co-composted biochar's slow release of essential plant nutrients like NO_3^- and $PO_4^{3}-$ (Kammann et al., 2015). Indeed, when placing pristine biochar, i.e. with empty nutrient reactive sites, into fertilized soil, the system's equilibrium will be in the direction of the nutrients binding to the nutrient-poor biochar, thereby adding a competitor for nutrients in the soil-plant system. But thanks to the charging process, biochar's nutrient reactive sites are already loaded. Therefore, when it is added to the soil, the system will either be at equilibrium in relation to nutrients or, if the external nutrient concentration is low enough, the nutrient-loaded biochar may release nutrients. Whether or not the released nutrients are available to plants, depends on the soil (texture, water content, pH, pore arrangement, organic carbon) and mineral coating of the biochar, but plants can create a depletion zone and hence invoke a gradient to access the nutrients either from the biochar or the compost (Joseph et al., 2018).

Biochar application rates

An important aspect to evaluate when considering the use of biochar as a soil amendment, is its application rate. As mentioned earlier, biochar can be made from any organic material, but the ideal feedstock should be biomass that can't or wouldn't be valued otherwise, like waste issued from wood, agricultural or food industry. All though several studies demonstrated biochar's beneficial effects when added to a cropping system on soil CEC, bulk density, water retention capacity, pH and fertility, these often consider application rates ranging from 10 t/ha to 100t/ha (Ding et al., 2016; Lévesque, Oelbermann, and Ziadi, 2022). These high application rates often exceed the availability of biochar feedstocks, considering raw material should be as local as possible and available at a reasonable price (Saba et al., 2023). Therefore, applications rates above 10t/ha aren't economically realistic (Hagemann et al., 2017). Indeed, we should avoid falling in a production scheme using additional limited land, water, and economical resources to grow biochar feedstock, which would partially, if not fully, cancel the counterbalancing effect biochar has on GHG emissions (Searchinger et al., 2008).This is why evaluating the quantity and long-term availability of a specific type of biomass is probably one of the most important aspects to cover when considering the use of biochar in a specific region, in order to then determine realistic application rates.

Biochar feedstock in British Columbia

When searching for a potential pyrolysis feedstock, wood waste is one of the first ideas that comes in mind considering that among provinces in Canada, British Columbia accounts for more than 50% of the production value of the whole primary wood manufacturing sector on national scale (Salehirad and Sowlati, 2007). By-products of this industry are already well exploited by multiple sectors (figure 7),this knowing that BC's recent government policies and programs encourage to increase utilization of onsite and roadside harvest waste, already counting these sources as part of the residue stream (Dobb and Hackett, 2021).

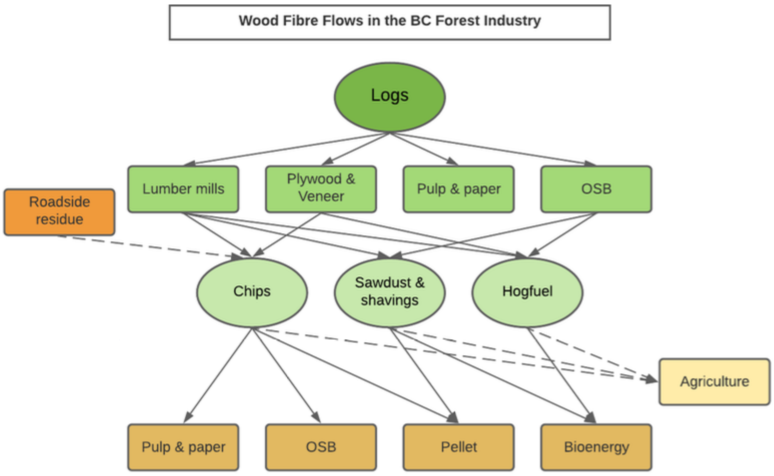

Figure 7: Wood fiber flows in BC's forest industry (Source: Dobb and Hackett, 2021)

Even though the agricultural sector has long been thought of as a secondary beneficiary, only using the surplus by-products left after all the demand from other consuming sectors was filled, wood waste's availability is truly critical for the functioning of BC's farming systems. Indeed, livestock bedding and poultry litter is one of the primary uses of sawdust and shavings (SDS). But because of an increase in mountain pine and spruce beetle infestations, devastating wildfires, contractual arrangements with pellet plant companies and reductions to the timber harvesting land base, BC's forest industry had to reduce its annual allowable cut, timber harvests and mill closures over the last two decades. This decrease in activities explains the increasing pressure the residual fiber market is facing. Recent models predict that supplies of SDS will continue to be constrained and competitive conditions will continue to intensify (Dobb and Hackett, 2021). One could think that adding biochar production on top of all the other already existing applications would only enhance the problem, but this might not be the case considering most of the field studies related to biochar typically involve one-time application (Shrestha et al., 2023). Moreover, if we remember the fundamental idea of producing biochar with locally available organic waste without any other way of valorization, a potential solution would be to pyrolyze the used livestock bedding and poultry litter. This way, the feedstock is present on site of use, with a guaranteed long-term availability and similarity.

Co-application : a potential new best management practise

The combination of several previous findings suggest that biochar-amendment practices deserve consideration as a new BMP to ensure temperate regions' cropping systems' nutrient requirements, while reducing their negative impact on the environment (Hagemann et al., 2017). Kammann et al. (2015)'s study demonstrated that co-composting improved biochar's plant growth promoting effects beyond the combination of pristine biochar with either mineral fertilizer or mature non-biochar-amended compost when applied on a *Chenopodium quinoa* W. crop in a sandy-poor soil amended with 2% (w:w) co-composted biochar. Given these results, the combination of biochar with non-pyrogenic organic matter seems to be the key strategy to develop carbon-fertilizer carriers that are effective at low application rates. Moreover, Saba et al. (2023)'s results suggest that combining nutrient-charged biochar with mineral fertilizer has potential in increasing crops nutrient-use efficiency. Therefore, if sufficient and affordable pyrolysis feedstock is available, co-applying conventional amendments with realistic doses of charged biochar could be one potential way of answering the necessity to reduce the amount of applied mineral fertilizers without affecting crop yields and nutritive status.

4 Objectives of the study

Altogether, the combination of a local opportunity for biochar production, co-application and charged biochar's previous proofs of efficiency along with the urgent need to reduce fertilizer use, make biochar-based amendment a realistic candidate as an alternative practice for British Columbia's agricultural landscape. Which brings us to the following question: can we reduce the amount of applied chemical fertilizer when co-applying it with charged biochar at micro-application rates without affecting a crop's nutrient status and yield?

To try and answer this question from an experimental point of view, a soil column greenhouse trial was conducted with as principal objective to assess the effect reducing the required amount of applied chemical fertilizer, when co-applied with charged hard wood biochar at micro-application rates (3t/ha), has on macro- and micronutrient's pool and crop yield characteristics of a spring wheat crop grown in a sandy acidic temperate soil. To try and respond to this objective, we proceeded by studying:

(a) **The effect of biochar-based fertilizers on soil properties** by observing soil's H_2O and $CaCl_2$-pH, total C and N content, and effective cation exchange capacity.
(b) **The effects of biochar-based fertilizers on crop nutrient status** by observing the above ground biomass's mineralomass, soil's bioavailable and exchangeable nutrients, and charged biochar physical structure and qualitative chemical composition through scanning electron microscope image analysis.
(c) **The relationship between soil properties, crop nutrient status and yield** by observing the above ground biomass and grain yield and calculating the harvest index, growth rate, critical dilution curves and N nutrition index.

II Materials and methods

1 Experimental design and treatments

A 88-day long greenhouse experiment was conducted in order to study the effect of reducing NPK fertilizer applications rates on nutrients phytoavailability when co-applied with biochar at micro-application rates.

25 soil columns were set up in a randomized bloc design (figure 8) where 5 different treatments where randomly distributed (table 1). Even though it would have been interesting to compare each application rate in presence and absence of biochar, given the available space in the greenhouse limiting the number of columns to 28, it was decided to privilege the number of replicas rather than the number of different modalities. The modalities that were chosen where those that allowed us to answer to the research question while being the most representative of what could be done one the field since no farming practice advises to reduce fertilizer applications rates without combining it with any other form of soil amendment.

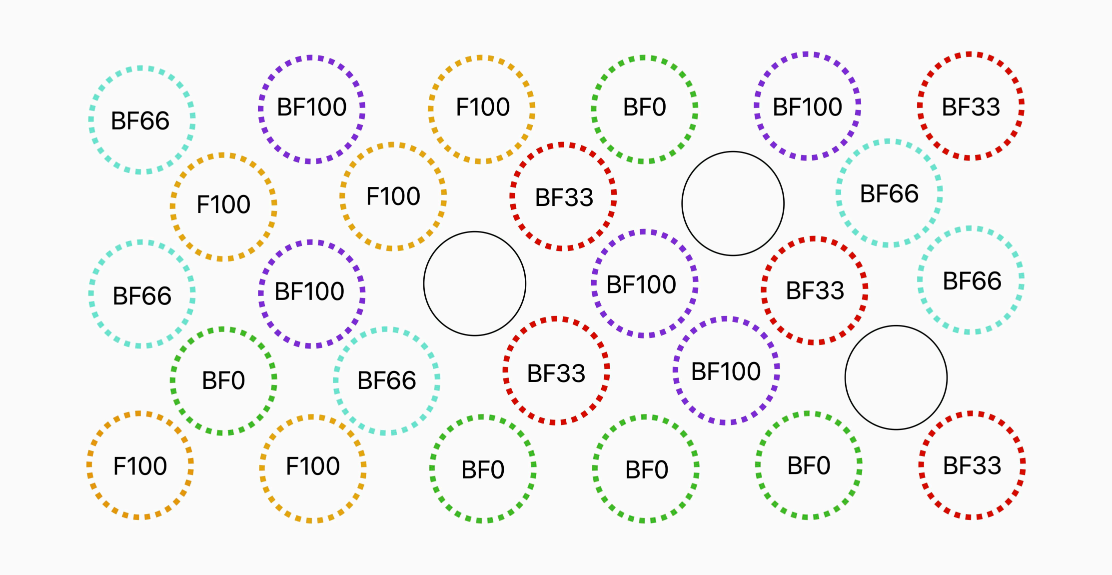

Figure 8: Experimental setup - randomized bloc design

Table 1: Experimental modalities and corresponding treatments

Modality	Treatment
BF100	100% NPK + biochar (3t/ha)
BF66	67% NPK + biochar (3t/ha)
BF33	33% NPK + biochar (3t/ha)
BF0	0% NPK + biochar (3t/ha)
F100	100% NPK

2 Crop

The experiment was conducted on spring wheat (*Triticum aestivum* L.) since it is the crop that is the most produced in Canada (Statistics Canada, 2022) and has a short growing cycle. Each column contained 9 spring wheat seeds sown 2cm deep and evenly distributed at the columns surface (circle of 7 and 2 in the center).

3 Soil amendments

i Biochar

The biochar used for this experiment was 2-6mm sized biochar from *BC Biocarbon*, made from residual forest wood at pyrolysis temperatures ranging from 600 to 800°C (table 2), appendix A for complete composition). It was charged by mixing dairy slurry to biochar with a 7:1 ratio in February 2022.

Table 2: Charged biochar total CNS content [%] and characteristics

	Charged biochar
N [%]	0.97 ± 0.04
C [%]	74.64 ± 1.67
S [%]	0.27 ± 0.02
H_2O-pH	10.2 ± 0.1
$CaCl_2$-pH	10.1 ± 0.1
Effective CEC	86.27 ± 8.56

In order to reproduce a 3t/ha application rate, knowing that the used biochar had a bulk density of 135kg/m^3 and that 1L of charged biochar weights 0.662kg, 11.55g of biochar were added to each column.

ii Fertilizer

A mixed fluid NPK chemical fertilizer (appendix B for complete composition) from *TerraLink* was used for this experiment.

The reference application rate of fertilizer was issued from the nutrient management calculator of the government of British Columbia [2]. This online tool allows BC farmers to easily estimate the amount of fertilizer they should apply on their field based on a soil sample (H_2O-pH, NO_3^-, P and K [mg/kg]), the cultivated plot (previous crops ploughed down, area, region) and co-applied amendments (manure, compost). After encoding the necessary information, the nutrient management calculator gave the crop requirements in terms of NPK presented in table 3.

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[2] nmp.apps.nrs.gov.bc.ca

Table 3: Reference fertilizer application rates issued from the nutrient management calculator of the government of British Columbia

	N	**P**	**K**
Crop requirement [lb/ac]	85	9	89
Crop requirement [kg/ha][3]	95.27	10.09	99.76
Crop requirement [L/ha]	68.05	7.20	71.25

Knowing that the used fertilizer had a ratio of 12-3-10 % of N, P, K respectively, and that in case of this soil, K is the limiting factor, the amount of fertilizer needed for each modality per column was defined as presented in table 4.

Table 4: Experimental fertilizer application rates and corresponding modalities

	BF100 & F100	**BF66**	**BF33**
Recommended [L/ha]	710	473	236
Per column [L/column]	0.557	0.372	0.186
Per application [mL/column]	0.186	0.124	0.062

All columns were amended through 3 distinct applications as advised by the 4R BMP to be synchronized with the peak demands of the developing plant [4] (table 5).

Table 5: Fertilizer application timing and corresponding growing stage

Date of application	**Growing stage**
1st March 2023	Establishment/tillering
22sd March 2023	Stem extension
11th April 2023	Grain filling

To evenly apply the fertilizer, each dose was collected with a micropipette (20-200μL) and diluted in 15mL of water before being poured directly at the surface of the column. Moreover, in view of the very coarse textured soil, special attention was paid to the moisture content of the soil when applying the fertilizer to avoid it being directly flushed through the column due to gravitational water flow.

4 Soil

The Land and Food System faculty possess a research field at the heart of UBC campus called Totem fields. It presents a wide range of soils, from rich and loamy to dry and sandy. The soil used in this experiment was extracted from the 0-30cm of a buffer area between two sandy parcels (figure 9), meaning no previous crops were grown on it and the soil didn't receive any previous treatments (fertilizer, pesticide).

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[3] Fertilizer density was assumed to be equal to 1,4 L/kg (Roy, 2006)

[4] www.yara.co.uk, March 2023

Figure 9: Soil extraction area - buffer area between tow sandy parcels of the UBC Totem Fields

Table 6 shows the analytical bulletin of the soil before the experiment. As mentioned in the introduction, biochar has shown great potential in coarse textured and acidic soil (Jeffery et al., 2017) explaining why soil for this experiment was extracted from this part of the Totem Field. Part of Totem Field's used to be an artificial golf field explaining the coarse texture, but no further specific soil classification can be given.

Table 6: Analysis bulletin of the experimental soil

pH H2O	pH CaCl2	effective CEC	Sand [%]	Loam [%]	Clay [%]	Available NO3- [mg/kg]	P [mg/kg]	K [mg/kg]
4.8	4.3	2.8	92	4	4	1.3 ± 0.2	70 ± 28	36 ± 5

To avoid any contamination, all of the extracted soil had to be autoclaved at 105°C and 100kPa for 30min before being brought to the greenhouse. Biocidal treatments like autoclaving remove or inhibit microbial activity which explains why several papers (Carter, Yellowlees, and Tibbett, 2007; Serrasolsas and Khanna, 1995) studied the effect autoclaving has on soil chemical and biological properties. One established fact is that by killing the active soil microbial biomass, autoclaving soil allows to exclude all influence of soil biology on soil processes (Carter, Yellowlees, and Tibbett, 2007). Thus, by autoclaving this experiment's soil, the effect of microbial activity on NPK dynamics has been excluded, putting the focus on soils chemical and physical components like biochar. Nevertheless, the charged biochar certainly brings back microbial activity to all the soil columns except modality F100, this will be taken into account when discussing the results.

Once the soil was autoclaved, it was oven dried at 105°C for 24 hours and sieved (<2mm) before building the columns.

5 Soil columns

20 columns (36cm high,10cm diameter) were filled with 4.175kg of soil and 11.55g of biochar according to the protocol in appendix C. The 5 columns of modality F100, considered as the reference treatment, were only filled with 4.175kg of soil (table 7 and figure 10).

Table 7: Charged biochar total CNS content [%] and characteristics

Volume	2827.4 cm3
Masse of soil	4175g
Masse of soil – first 21cm	2644g
Masse of soil – last 15 cm	1531g
Bulk density	1.48g/cm3
Masse of biochar	11.55g

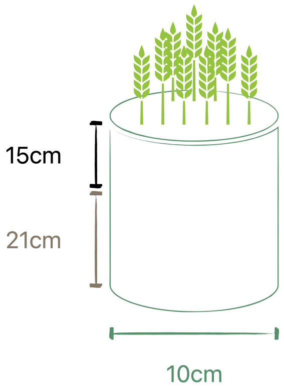

Figure 10: Soil column characteristics

All columns were equipped with an automatic drip irrigation system (irrigation pipe: JM Eagle Schedule 40 1 inch, drip assemblies: Netafim, supplier: Watertec) watering them twice a day every two days with 52.5mL of water during the 28 first days of the experiment, and with 52.5mL of water twice a day every day during the rest of the experiment (figure 11). On day 65, the irrigation system was shut down to let the wheat dry down before collecting the grains at maturity.

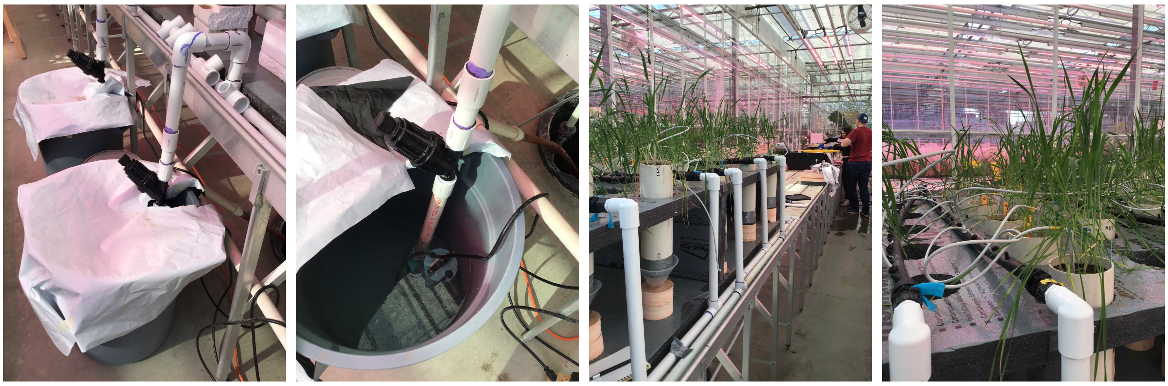

Figure 11: Automatic drip irrigation system

A failure in some parts of the irrigation system caused water stress for a number of plants at different stages of the experiment. Observations were made in an experimental logbook and were considered before treating the data and during the data interpretation.

The greenhouse average weekly temperature and relative humidity conditions are presented in table 8.

Table 8: Greenhouse average weekly temperature and relative humidity conditions

Week	Avg temp [°C]	Avg RH [%]
7	20.3	44
8	19.3	43
9	20.4	43
10	21.6	37
11	22.5	34
12	22.3	38
13	23	32
14	22.8	39
15	22.8	36
Avg	**21,7**	**38,4**

6 Laboratory analysis

Samples were analyzed at the soil res3 lab of the LFS faculty at UBC, by the analytical laboratory of the Ministry of Environment and Climate Change Strategy (MOE) of British Columbia or at the Greenmat research laboratory of ULiège depending on the analysis's nature and the available equipment.

i Plants

The above ground biomass of 5 out of 9 plants of every column were collected on the 40th day of the experiment, when the flag leaf sheath was beginning to open meaning the spring wheat was at growing stage 47 of Zadoks growth staging system (harvest 1, Z45) (figure 12). The plants were then oven dried at 105°C for 24 hours. Each collected plant biomass was then chopped with scissors in a little container to which 2 metal beads were added. Then all of the samples were put in a shaker machine (originally used to mix paint) for 15min turning the biomass into thin powder. The total weigh of each grinded material was measured using a 0.05g precision scale (ranger 7000 OHAUS scale) before sending part of the sample to MOE to analyze standard metal concentration.

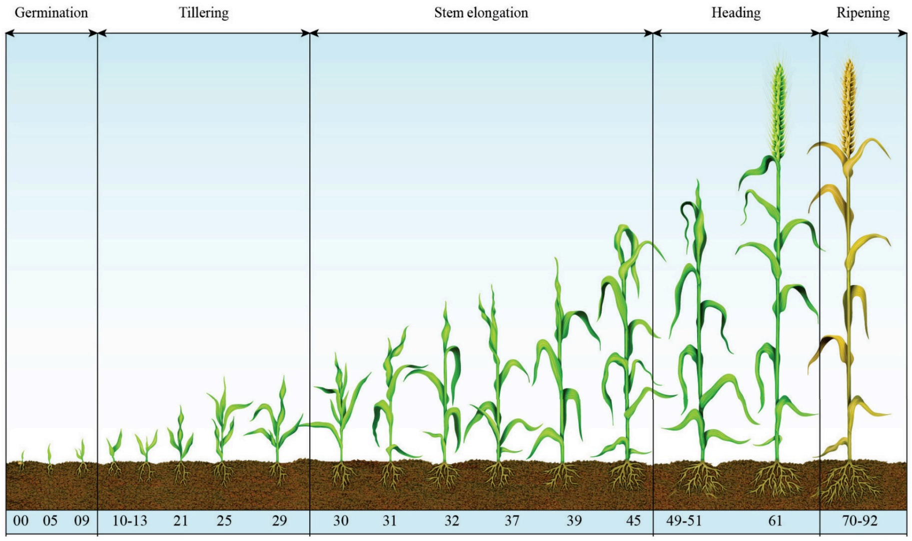

Figure 12: Zadoks growth statging scale for spring wheat (Source : Bengtsson, 2013)

Quantification of above ground biomass CHNS was done using a Thermoscientific FlashSmart elemental analyzer equipment. For each sample, between 2 and 3mg of dry soil were weighed using a 0.001 mg precision scale (Mettler Toledo XP6).

ii Grains

On the 88th day of the experiment, the above ground biomass of the 4 remaining plants of each column were collected (harvest 2). After being dried for 48 hours at 80°C (Darroch and Baker, 1990) the grains were separated from the rest of the plant and each part was weighted separately using a 0.05g precision scale (ranger 7000 OHAUS scale). The yield of each column was then converted to the total mass of grain per hectare [t/ha].

iii Soil

On the 40th day of the experiment, topsoil (0-3cm) was extracted from each column and stored in the fridge. For each replicate, 100mg of fresh soil was send to MOE and submitted to the analysis presented in table 9.

Table 9: Analysis conducted on the soil samples

	Extraction method	**Quantification method**
Available NH_4 and NO_3 [mg/kg]	2N KCl	Colorimetric
Exchangeable cations (Al, Ca, Fe, K, Mg, Mn, Na) and effective CEC [cmol$^+$/kg]	0.1N $BaCl_2$ extraction	Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES)
Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn [mg/kg]	Mehlich-3	Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES)
Total C and N [%]	-	Gas chromatographic method (Flashsmart elemental analyser)

Mehlich-3 is a weak acid soil extraction procedure commonly used in north America to estimate plant-available micro- and macronutrients in acidic soils (Nyiraneza et al., 2019). It is composed of 0.2 N glacial acetic acid $CH_3.COOH$, 0.25 N ammonium nitrate NH_4NO_3, 0.015 N ammonium fluoride NH_4F, 0.013 N nitric acid HNO_3, and 0.001 M ethylene diamine tetra acetic acid (EDTA) combined at pH 2.5 (Mehlich, 1984).

The rest of the soil samples where oven dried during 24 hours at 60°C before being sieved at 2mm. 10mg of dry soil were put in a centrifuge tube to which 20mL of distilled H_2O or $CaCl_2$ (0,01M) were added to quantify soil H_2O and $CaCl_2$-pH respectively. The tubes were then put in a shaker table for 30 minutes before measuring H_2O and $CaCl_2$-pH using a pH reader. The pH meter was calibrated every 25 readings and the pH probs was rinsed with distilled water between each sample.

Quantification of soil's total C and N content [%] was done using a Thermoscientific FlashSmart elemental analyzer equipment. For each sample, between 50 and 100mg of dry soil were weighed using a 0.01mg precision scale (XSR105 Mettler Toledo scale).

All sample will be compared to baselines corresponding to soil samples collected at the Totem field in May 2022 before a previous spring wheat field experiment that took place on the parcel directly next to the zone where this experiment's soil was extracted. These samples were submitted to the exact same quantifications according to the same protocols (H_2O and $CaCl_2$ pH) or executed by the same lab (CEC, available ammonium, nitrate and perform a Mehlich III extraction done by MOE).

iv Biochar

The physical structure and qualitative chemical composition of pristine and charged biochar were observed and compared using a TESCAN CLARA scanning electron microscope (SEM) at the Greenmat research laboratory of ULiège. SEM analyses were carried out using an acceleration voltage of 15keV with a working distance of 10 mm. For each type of biochar, 3 samples were randomly collected and fixed on an aluminum plate using double-coated carbon conductive tape before being covered with carbon using a SPI Modul sputter Coater.

Both types of biochar were observed according to the same protocol through 2 distinct seances of 3 hours each. The 3 samples were observed through ET lenses and 4Q BSE-compo. When the BSE-compo lens revealed particles or parts from a different tone than the rest of the structure, a qualitative chemical analysis was performed on the concerned spot.

7 Crop yields

To assess the effect of the different modalities on a spring wheat crop physiology, 3 indicators were calculated.

i Harvest index

The harvest index [%] is a ratio that represents the proportion of the harvested yield (grains) to the total biomass produced by a crop (stem and leaves) and reflects the efficiency of a crop in converting photosynthetic products into harvested products. For each column, the ratio between the dry grain mass and shoot dry mass of harvest 2 (Z88) was calculated.

ii Growth rate

The growth rate of each column was established by calculating the difference between the shoot dry mass at harvest 2 (Z88) and harvest 1 (Z45).

iii Nitrogen critical dilution curve and nutrition index

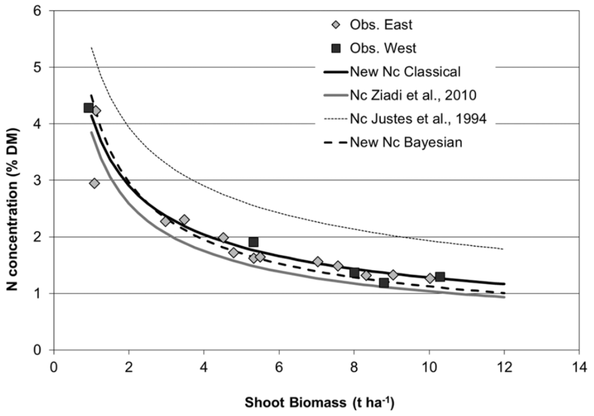

Figure 13: Critical nitrogen (N) dilution curves using data from spring wheat grown in Canada. The solid line (New Nc Classical) represents the critical N curve found by Jégo G. et al. (2022) (Nc = 4,14 W–0.51) with the classical approach of Justes et al. (1994) along with published curves and the new Nc curves defined with the Bayesian approach proposed by Makowski et al. (2020).

A nitrogen critical dilution curve (CDC) (figure 13) is the relationship between a crop's dry shoot biomass [t/ha] and the total concentration of nitrogen [% of dry weight] at a given time during the crop cycle (Jégo et al., 2022). The general relationship can be described by the following equation:

$$[N] = a.W^{-b}$$

Where W is shoot dry matter [t/ha], *[N]* is the total nitrogen concentration in the biomass [%] and a and b are constants that vary between crops nutrient uptake situations. The values for a and b for a spring wheat crop were those found by Jégo et al. (2022) and are presented in table 10.

Table 10: Charged biochar total CNS content [%] and characteristics

	a	**b**
$[\mathbf{N}]_{minium}$	1.3	0.40
$[\mathbf{N}]_{critical}$	4.14	0.51
$[\mathbf{N}]_{maximum}$	6.7	0.51

A CDC reflects the concept that every crop has its minimum essential nitrogen content requirements, called critical concentration, to support an optimal growth and yield bellow which a crop will be considered in deficiency and above which a crop will be considered in luxury-consumption. Indeed, when nitrogen concentrations are low, increasing the nitrogen availability leads to improved crop growth and productivity. However, as nitrogen concentration increases, the crop's demand for nitrogen diminishes, and the additional nutrient may not increase the crop's productivity performances anymore.

The nitrogen nutrition index (NNI) [%] is the ratio between the actual concentration and the critical concentration of a crop for nitrogen. Values of the index below 1 indicate a nitrogen deficiency while values above 1 indicate a nitrogen luxury uptake.

8 Statistical analysis

All statistical analyses were performed on Rstudio. All samples were assumed to be random, simple, and independent. Statistical analyses on all parameters were conducted according to the scheme presented in figure 14.

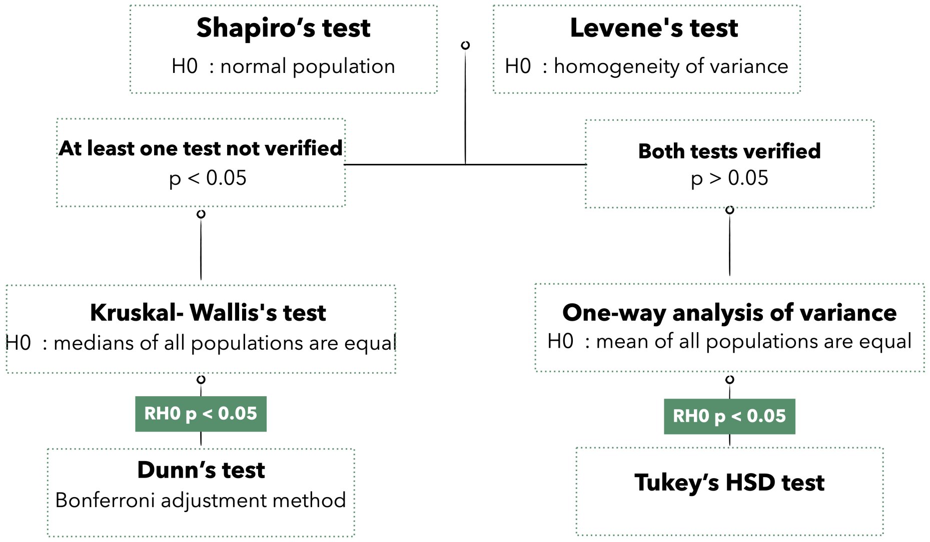

Figure 14: Statistical analysis protocol

Due to manipulation errors during the weighting of the biomass samples, the exact mass of sample 16BF33 and 19BF0 couldn't be established. It was decided to suppress these samples of the mineralomass data to avoid any false interpretations. Knowing that water limitations can strongly affect a crop's yield, the yield data of the second harvest from samples 4BF0,5BF100, 6BF33, 27BF0 and 28BF33 were also not considered since they had been exposed to severe hydric stress due to an unsolved problem in the irrigation problem (occurred after harvest 1, starting from day 50^{th} day of the experiment). Finally, all sample quantifications under a given detection limit were not considered and set as *not applicable* (NA).

III Results

The nutrient related results are synthesised in table 11 and will be presented graphically if the the nul hypothesis of equality of Tukey or Dunn's test was rejected at alpha=0.05. Detailed numerical results can be found from appendix D to H.

Table 11: Recapitulating table of nutrients related data

	Soil exchangeable [mg/kg]	**Soil bioavailable [mg/kg]**	**Biomass mineralomass [mg]**
N	|	=	=
P	|	=	=
K	BF0 <BF100 and BF66	BF0 <BF100	BF0 <BF100 and BF66
S	|	BF0 <BF33 <BF66 <F100 <BF100	BF0 <all modalities
NH4+	|	BF0 <F100	|
NO3-	|	=	|
Al	BF0 >F100 and BF100	=	=
B	|	<DL	=
Ca	BF0 <all modalities	BF100 <F100,BF100 and BF66	BF0 <F100
Cu	|	=	BF0 <BF100 and BF66
Fe	=	=	=
Zn	|	=	F100/BF0 <BF100 and BF66
Mg	BF0 <BF100 and BF66	=	BF0 <BF100 and BF66
Mn	=	=	=
Na	=	=	=
Mo	|	|	=

Note : "|" no data ; "=" no significant difference by Tukey or Dunn's test at α=0.05, ">" or "<" significant difference by Tukey or Dunn's test at α=0.05, "<DL" under quantification detection limit

1 Soil

i H_2O and $CaCl_2$ pH

Figure 15 presents H_2O (A) and $CaCl_2$ (B) soil pH of the baseline and modality F100 to BF0.

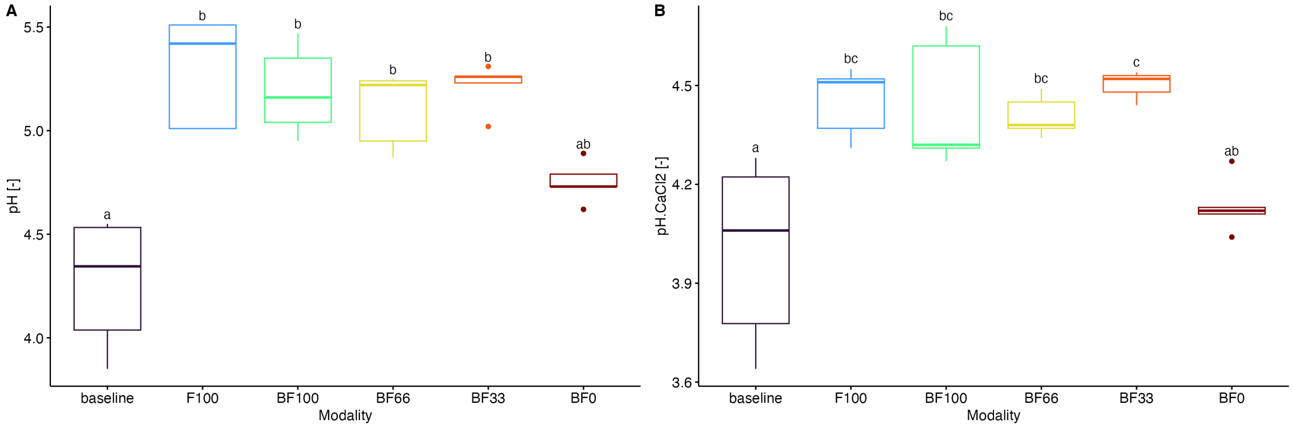

Figure 15: H_2O (A) and $CaCl_2$ (B) soil pH

ii Total N and C content

Figure 16 presents soil total N (A) and C (B) content [%] after harvest 1. No significant difference came out of the statistical analysis, all modalities can therefore be considered equal.

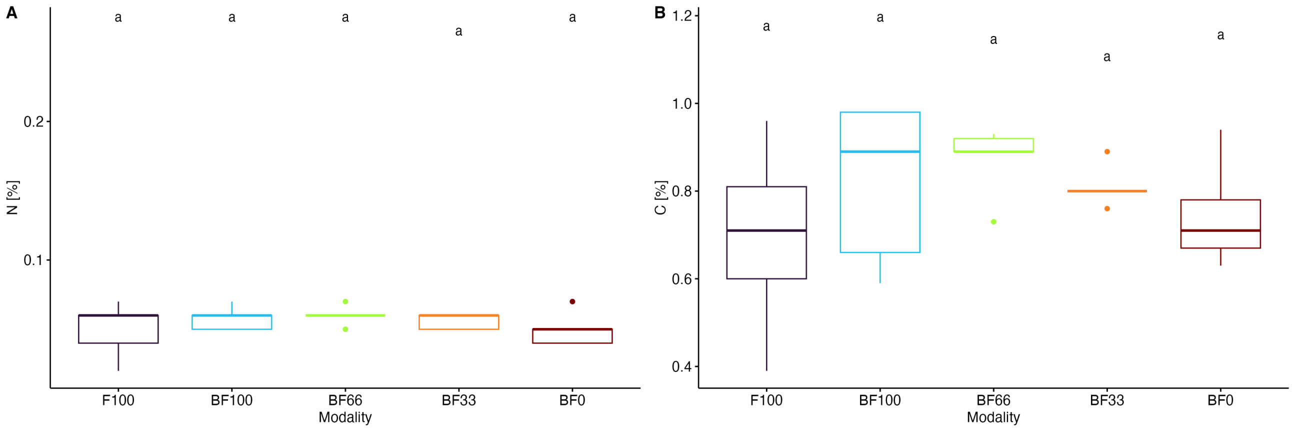

Figure 16: Soil total N (A) and C (B) content

iii Exchangeable elements and effective CEC

Figure 17 presents the exchangeable elements (A to D) and effective CEC (D) [cmol$^+$/kg] of the baseline and modality F100 to BF0.

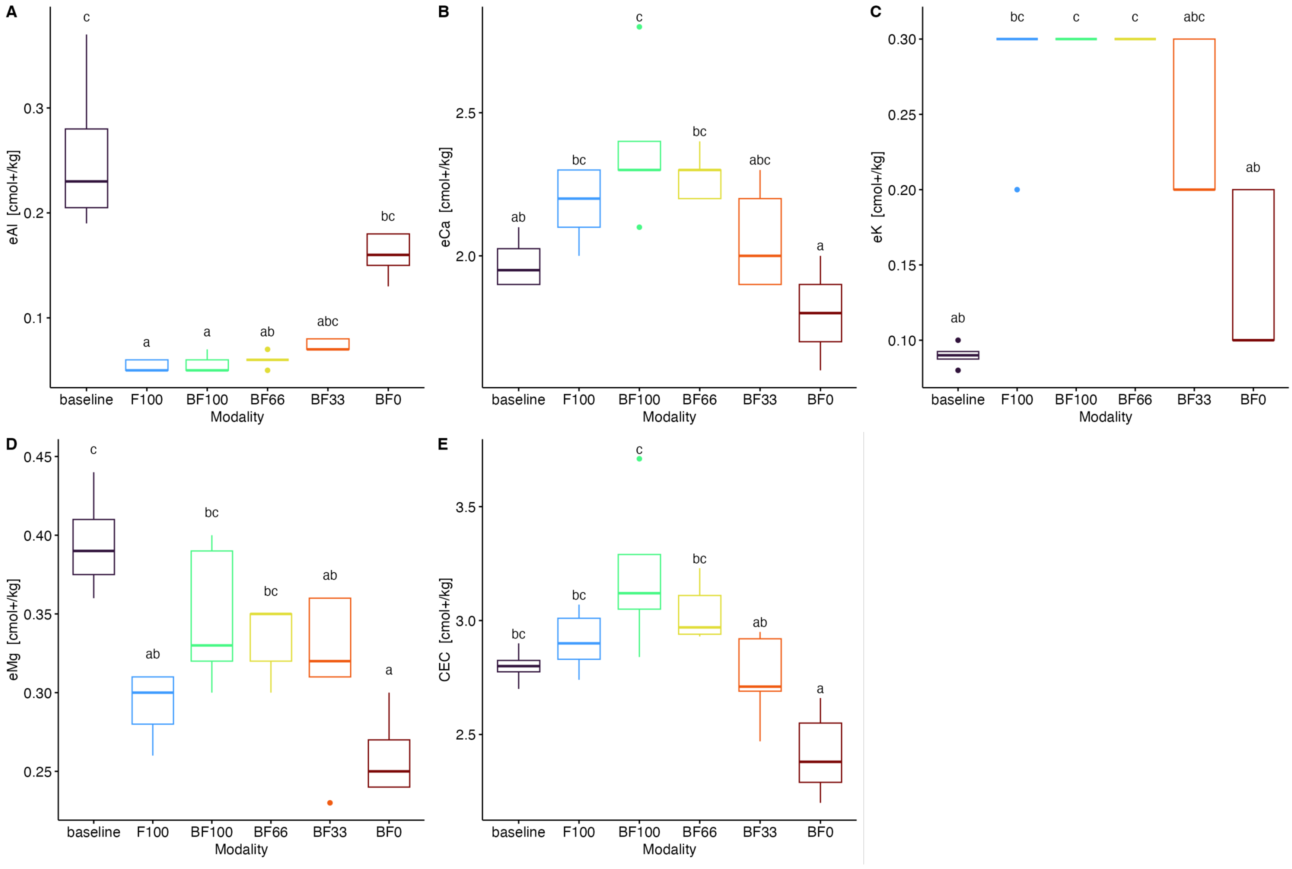

Figure 17: (A) eAl (B) Ca (C) eK (D) Mg (E) effective CEC [cmol$^+$/kg]

iv Ammonium, Nitrate and bioavailable nutrients content

Figure 18 presents NH_4^+ and soil's bioavailable nutrients content [mg/kg] quantified via Mehlich III extraction of the baseline and modality F100 to BF0 for which the nul hypothesis of equality of Tukey or Dunn's test was rejected at α=0.05.

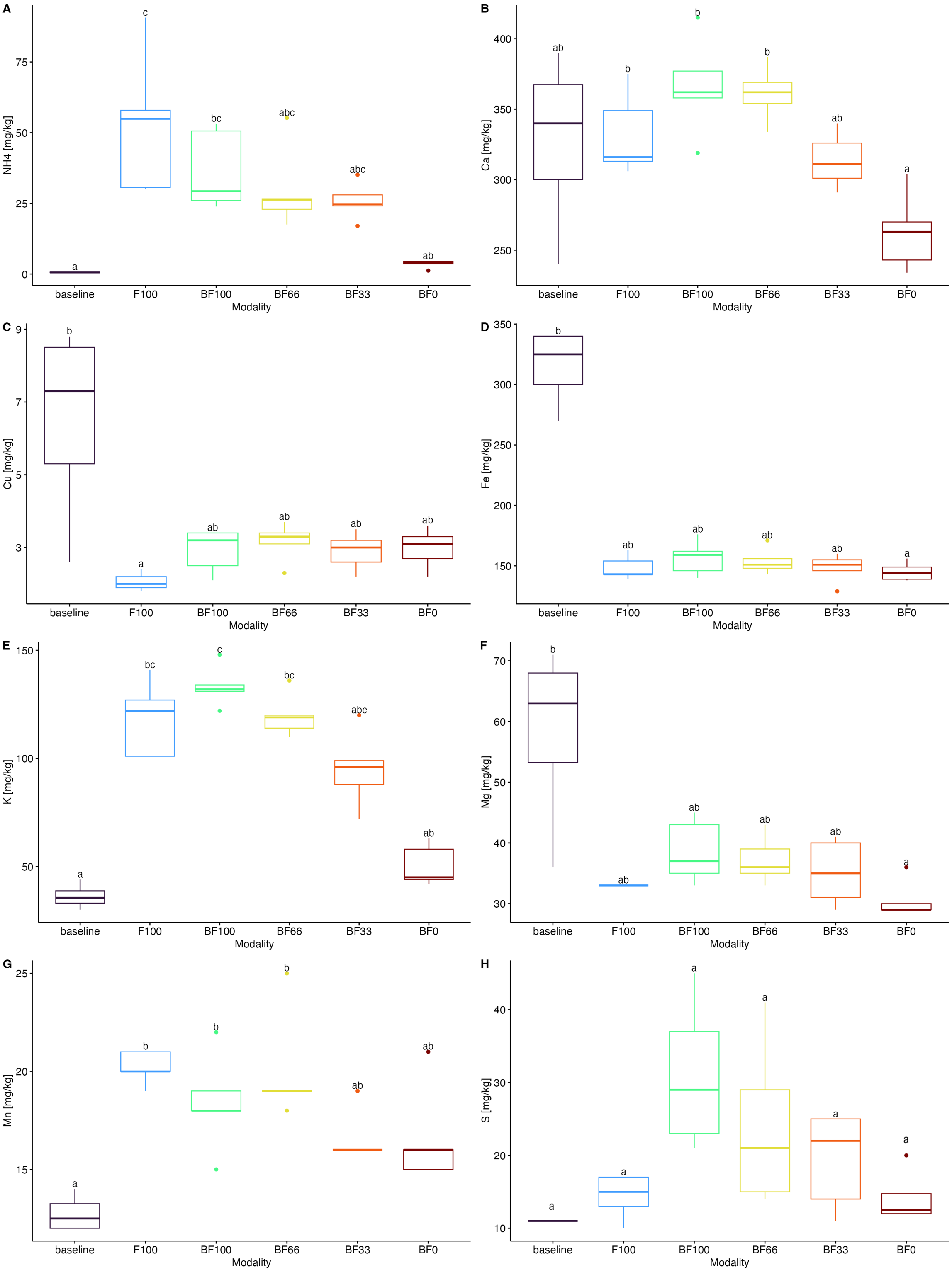

Figure 18: Soil (A) NH_4^+ (B) Ca (C) Cu (D) Fe (E) K (F) Mg (G) Mn (H) S content [mg/kg]

2 Biochar SEM imaging analysis

i Physical characterization

Two main physical structures are observed on pristine biochar. The left side of figure 19 a presents a tubular structure in opposition to the very irregular and shattered structure that can be observed on the right side. This tubular and more regular shapes can also be observed at a bigger magnitude on figure ref e. The general physical structure of charged biochar is more diverse as can be seen on figure ref b. Tubular zones (figure ref 1.b) coexist with more texturized and irregular zones (figure ref 3b and f) along with more complexe structures (ref 2b). The transversal cut of charged biochar on the right side of figure ref d, reveals the regular tubular structure. Small circular holes can also be observed inside the tubular structure of pristine biochar but through a lateral cut (figure ref c).

ii Qualitative chemical characterization

All spectrums, whether of pristine or charged biochar, present a pic around 0.25 and 0.5 keV indicating the presence of C and O respectively (figure 20 and 21 a to g).

Figures 20 a to c are issued from the observation of pristine biochar. Two similar particles (figure 20 1a and 2a) that contrast with the rest of the pristine biochar, present the same spectrum indicating the presence of Ca, Fe, Mg, Si and Al. Figure 20 b shows a water droped shaped particle and it's spectrum presenting 2 important pics of Fe around 1.75 and 6.5 keV. Figure 20 2c is a zoom on one of the white particules that can be observed on figure 20 1c and it's spectrum that revealed the presence of Na and Ca.

Figures 21 d to g are issued from the observation of charge biochar. Figure 21 d shows small lighter aggregates and their spectrum presenting a significant pic of Ca. Almost perfectly shaped circularly shaped aggregates were found repeatedly at the charged biochar's surface as can be observed on figure 21 f. They globally presented similar spectrums with a high pic of Mg, P and Ca as can be seen on the spectrum of particle 21 1f and 2f. As shown on figure 21 g, some very contrasting elements were found. The spectrum of the mound of particles (upper left) presents pics of Mg, Si, K and Na. The thread shaped particles were too narrow to give relevant spectrums, only C and O pics were observed.

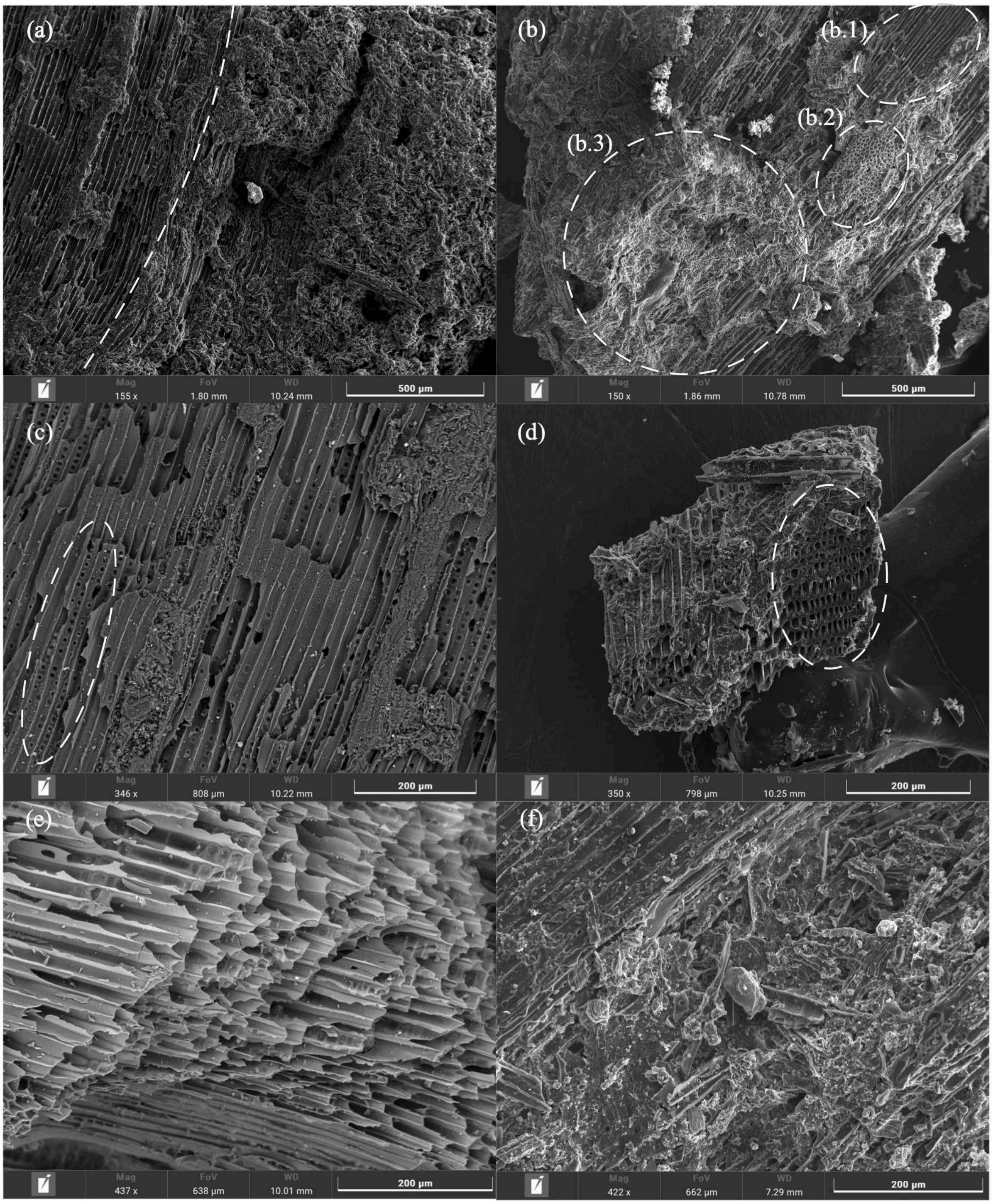

Figure 19: 15 keV SEM imaging of (a) E-T pristine biochar (b) E-T charged biochar (c) pristine biochar BSE-compo (d) E-T charged biochar (e) pristine biochar BSE-compo (f) E-T charged biochar

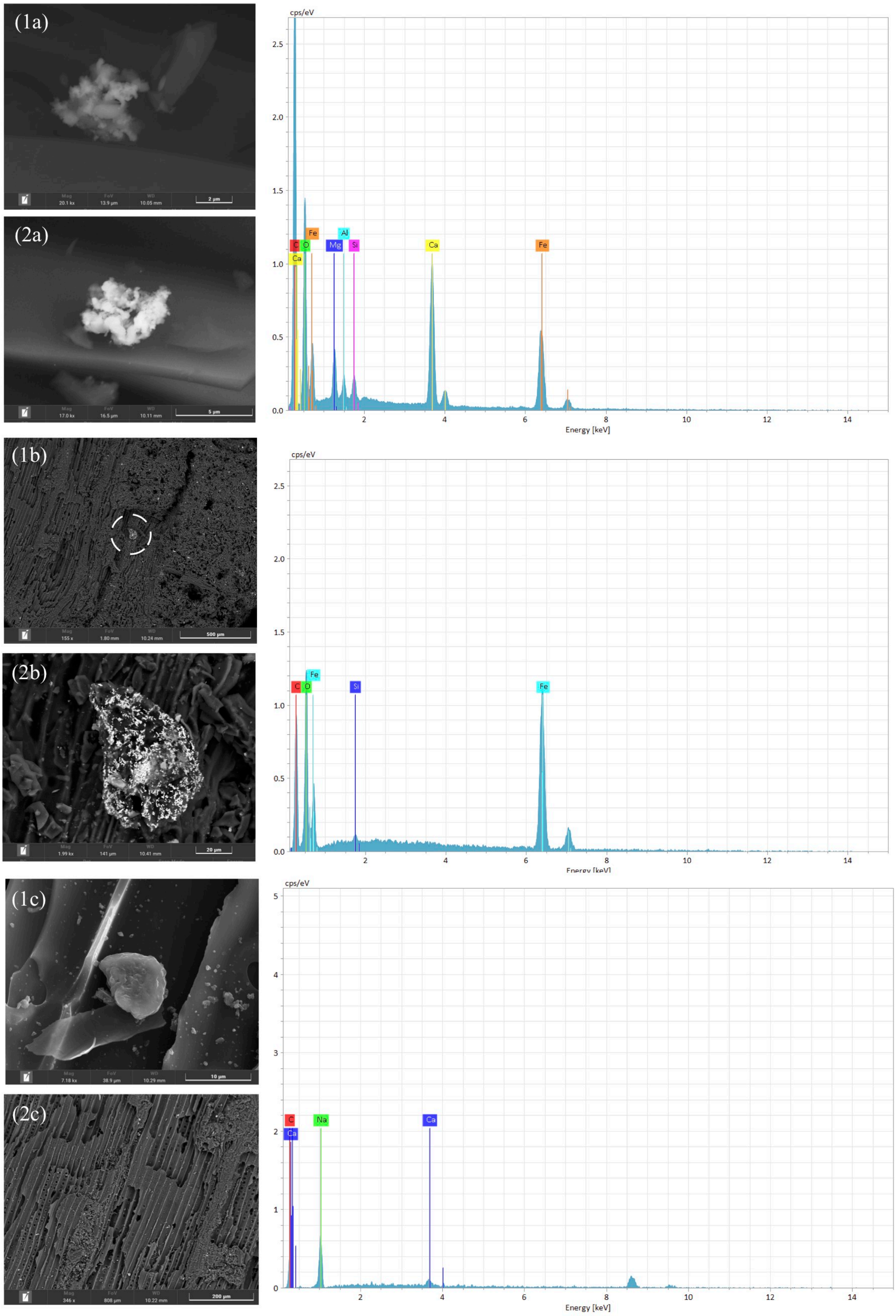

Figure 20: 15 keV SEM imaging of (1a) pristine biochar BSE-compo (2a) pristine biochar BSE-compo (1b) pristine biochar BSE-compo (2b) pristine biochar BSE-compo (1c) pristine biochar BSE-compo (2c) E-T pristine biochar

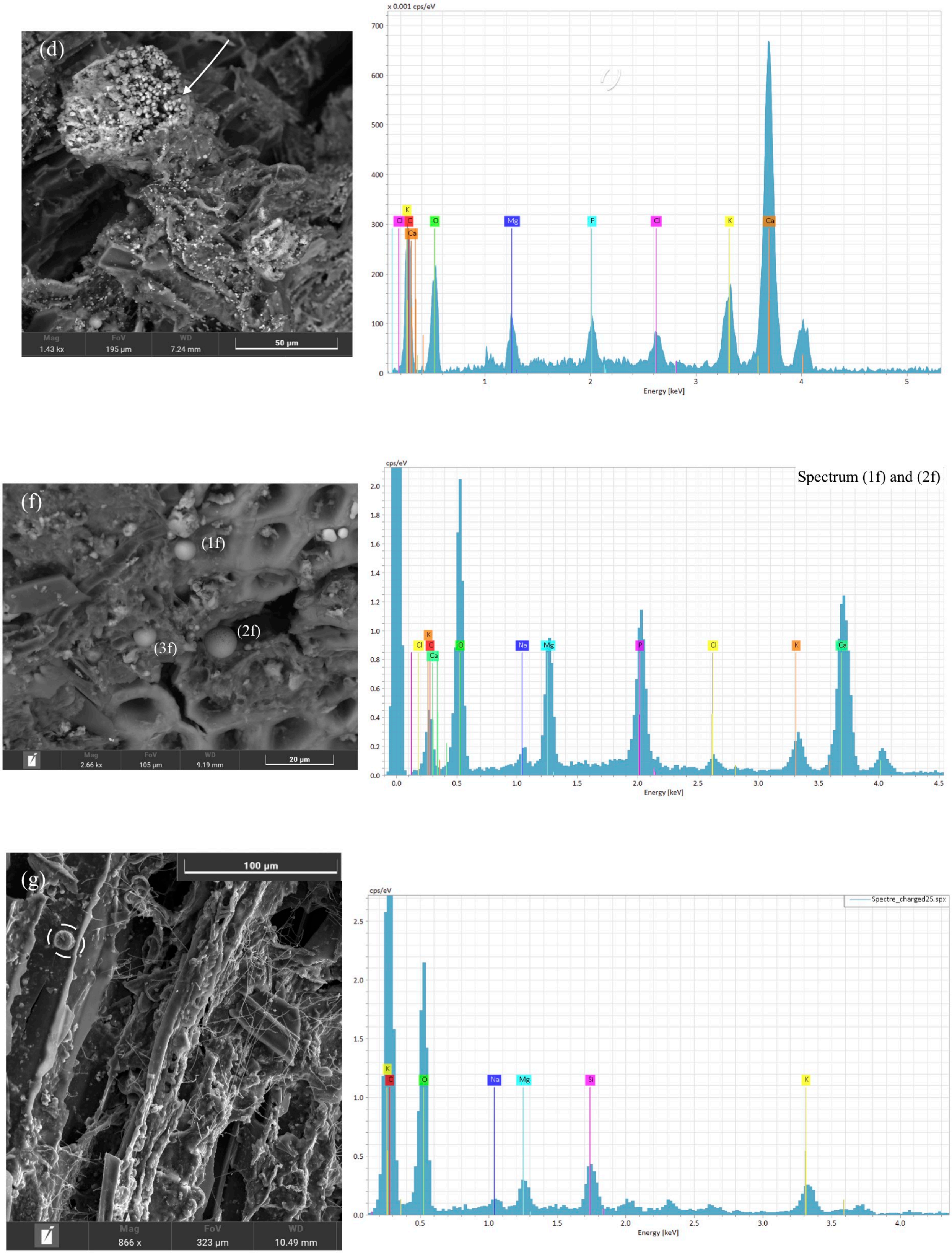

Figure 21: 15 keV SEM imaging of (d) charged biochar BSE-compo (f) charge biochar BSE-compo (g) E-T charged biochar

3 Biomass

i Biomass mineralomass

Figure 22 presents the above ground biomass's mineralomass [mg/column] of modality F100 to BF0 in Ca, Cu, K, Mg, S and Zn for which the nul hypothesis of equality of Tukey or Dunn's test was rejected at α=0.05. All other samples passed the tests and can there for be considere as equal.

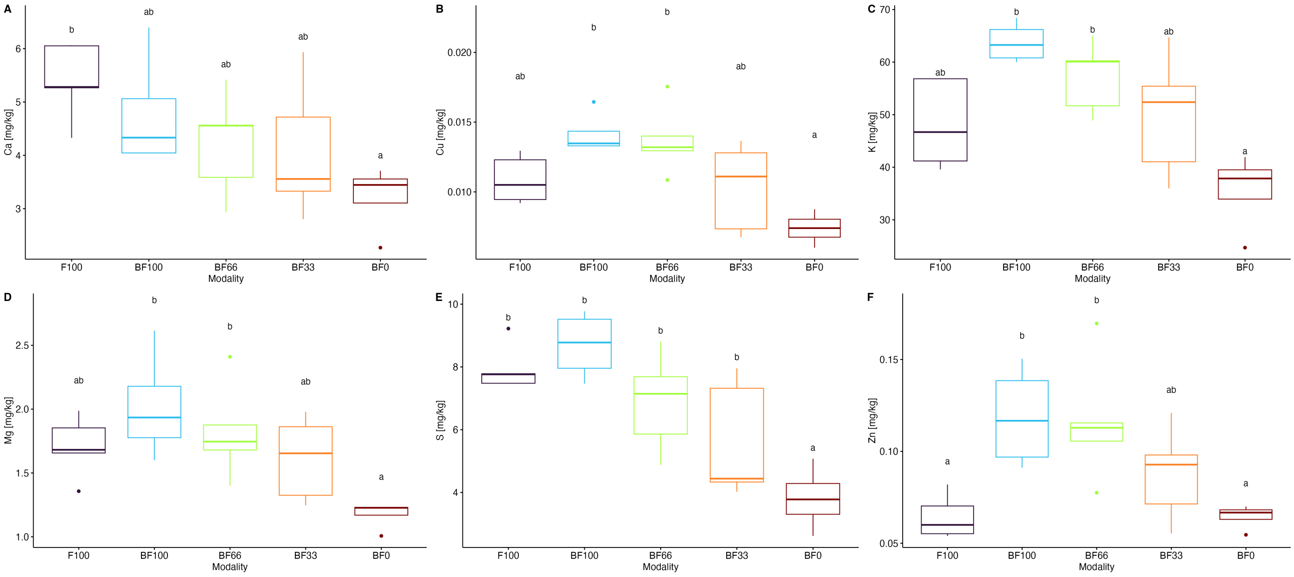

Figure 22: Above ground biomass (A) Ca (B) Cu (C) K (D) Mg (E) S (F) Zn mineralomass [g/column] at harvest 1 (Z45)

ii Total N and C content

Figure 23 shows total N and C content [%] of the above ground biomass collected at harvest 1. Only total N content of modality BF0 was significantly lower than modality F100, all other samples can be considered equal.

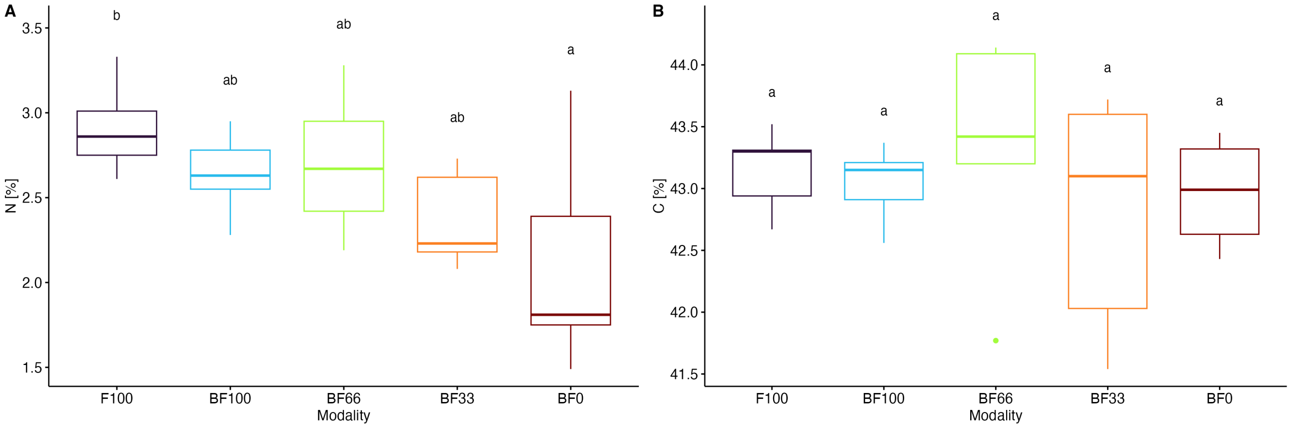

Figure 23: Total above ground biomass (A) N content (B) C content [%]

iii Nitrogen critical dilution curve and nutrition index

Figure 24 presents all samples organized per modality in spring wheat's N critical dilution curve. BF0's N nutrition index (NNI) is significantly lower than all other modalities (table 12), all other treatments can be considered equal.

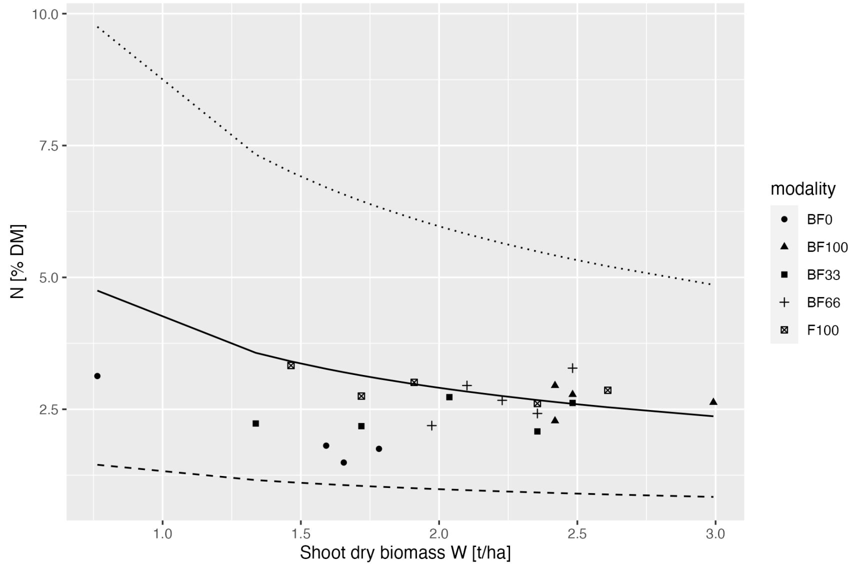

Figure 24: Spring wheat N CDC with samples from modality BF0 to F100

Table 12: N nutrition index

	NNI
F100	1 ± 0,1 b
BF100	1 ± 0,1 b
BF66	1 ± 0,2 b
BF33	0,8 ± 0,2 b
BF0	0,6 ± 0,1 a

Note : modalities with the same letter are considered equal by Tukey or Dunn's test at α=0.05

4 Crop characteristics

i Biomass and grain yield

Figure 25 presents harvest 1 (A) and 2 (B) stem and leaves yield [t/ha] as well as the grain yield (C) [t/ha] and total number of grains per column (D) from modality F100 to BF0. At harvest 1, BF0' yield is significantly lower than modality BF100. All other samples can be considere equal.

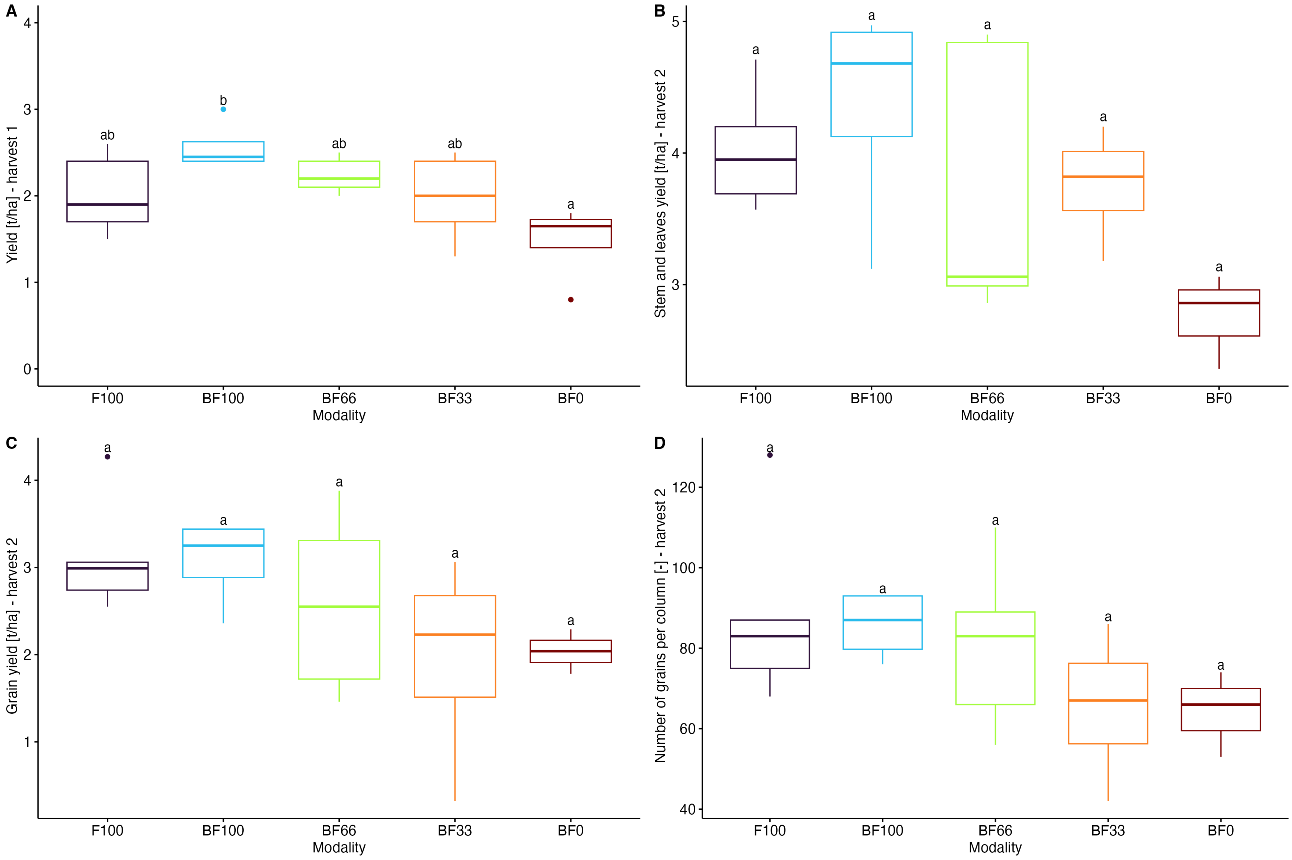

Figure 25: (A) Total above ground biomass yield [t/ha] at harvest 1 (B) Stem and leaves yield [t/ha] at harvest 2 (C) Grain yield [t/ha] at harvest 2 (D) Number of grains per column [-]

ii Harvest index and growth rate

Graph A and B (figure 26) present the harvest index [%] and growth rate [g] of modality F100 to BF0 respectively. The nul hypothesis of Tuckey or Dunn's test at $\alpha = 0.05$ is accepted for both parameters, meaning there is no significant difference between the different modalities.

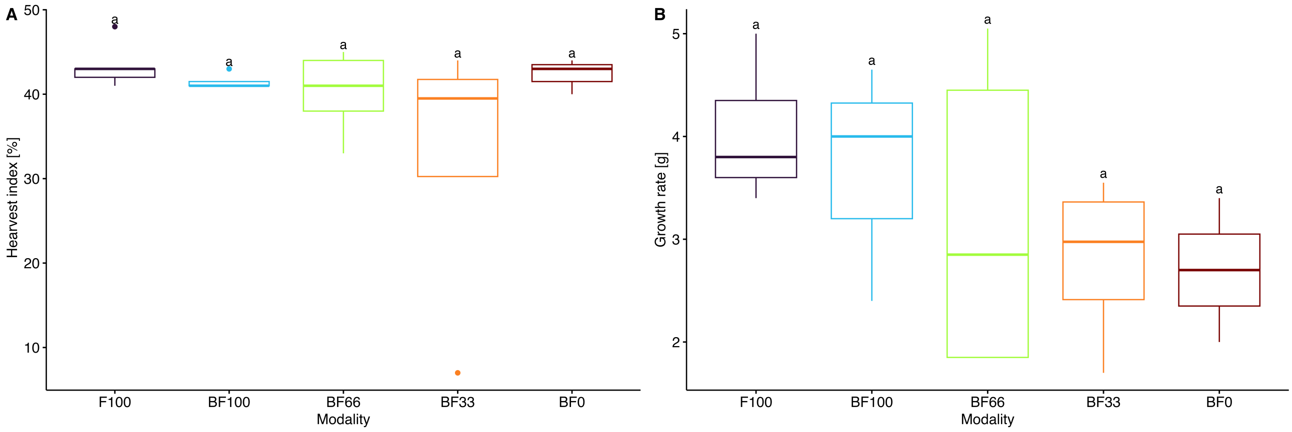

Figure 26: (A) Harvest index [%] (B) Growth rate [-]

IV Discussion

Effect of biochar-based fertilizers on soil properties

No significant differences could be observed between H_2O and $CaCl_2$ soil pH of modalities with (BF100, BF66, BF33, BF0) and without (F100) charged biochar (appendix D). Considering the biochar raw material used in this experiment, it could be that the micro-application rate of 3t/ha is simply too low to induce changes in H_2O and $CaCl_2$ soil pH. Indeed, the liming capacity of biochar is related to its chemical composition, specifically to its content in alkaline minerals, such as calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). The release of base cations ($Ca_2{}^+$, Mg_{2+}, $Na_2{}^+$) and carbonates ($CaCO_3$, $MgCO_3$, $NaCO_3$) contributes to the neutralizing effect on soil acidity (Chintala et al., 2014). With this is mind, taking into account that the utilized hardwood biochar presented a Ca and Mg content of only 4.8 and 2.2g/kg respectively (appendix A) and considering its micro-application rate, the alkaline minerals input was low, resulting in a correspondingly limited liming effect.

This hypothesis is consistent with the results of an experiment led by Chintala et al. (2014). They studied the effect of corn stover and switchgrass biochar addition on the pH of an acidic soil. Both biochar's also presented a low Ca content of around 7.5 and 7.2 g/kg respectively, but in contrast with the present study, were experimented at 3 very high application rates (52, 104, and 156 t/ha). As a result, soil pH was significantly increased following the application of both biochars despite their low alkaline minerals content. Moreover, they observed that the magnitude of the pH increase was positively correlated with biochar's application rate and Ca content, indicating a positive dose-dependent relationship between the applied biochar doses, its Ca content and soil pH. The present studies' hard-wood biochar with limited liming capacities, combined with a very low application rate, could therefore explain the absence of significant variation in soil pH following its application.

The low application rate can also explain why, despite the high carbon composition of biochar, no increase in soil C_{tot} was revealed following its addition to soil (figure 16. Adding 11.55g of charged hard-wood biochar (75% C content) in the upper 15cm of the soil columns, only represents a 0.57% increase of the considered soil mass and was probably not significant enough to detect and measure a significant difference in soil C_{tot}.

These results agree with Schulz, Dunst, and Glaser (2013) greenhouse experiment that studied the effect of co-composted biochar on oat (*Avena sativa* L.) in a sandy soil when applied at rates between 0.03 and 2.5 t/ha. They didn't observe any effect of biochar addition on pH or total organic carbon and, in accordance with the previously outlined hypothesis, they explained this by the extremely low application rates that were tested.

Nevertheless, when compared graphically, C_{tot} decreased from modalities BF100 to BF0 according to the fertilizer application gradient and the median value of F100 was lower than BF100's median even if not significantly (figure 16B). The same trend can be observed on soil H_2O pH, exchangeable (eCa) and bioavailable (Ca_{bio}) Ca graphics (figure 15A, 17B and 18B). A lower Ca content can explain why compared to baseline, all treatments except BF0 presented a significantly higher H_2O and $CaCl_2$ soil pH. Due to the more alkaline environment, stabilized carbonates ions could have reacted with cations to form carbonates compounds, slowing down carbon decomposition and thus, increasing, even if not significantly, soil's C_{tot} content in treatments that included mineral fertilization (Bughio et al., 2016). Additionally, modalities that presented a higher biomass production could have beneficiated off higher carbon inputs into the soil through root exudates (Panchal et al., 2022).

Besides, a lower pH can cause retention of positively charged hydroxyl-Al polymers on the soil exchange complex potentially explaining why the exchangeable Al_3^+ represented 1.9% of modality BF100's effective CEC against 8.3% of modality BF0's (appendix **??**) (Chaplain et al., 2011).

On the whole, soils properties were thus not significantly impacted by the charged biochar input. This agrees with previous studies' results and is probably due to the nature of the raw material combined with its low application rate.

Biochar-based fertilizers' effect on crop nutrient status

After 45 days of growth, the sole application of charged biochar (BF0) significantly reduced Ca and S mineralomass [mg] compared to the conventional treatment (F100) (appendix H). Clearly, against biochar's 12.4 mg S input, fertilizer's 52.6 mg S input was a non-negligible contribution that the modality BF0 was deprived off (annex I). Despite this, after harvest 1, S_{bio} of all modalities were equal (appendix F). Unlike S, charged biochars' Ca input was significantly higher than fertilizer's Ca input (annex I). Despite this, modalities that received fertilizer presented higher Ca mineralomass than BF0 (appendix H). This could be due to a difference between the forms in which Ca was more or less easily available to the plants. When Ca was imported through fertilizer application, Ca was directly contained in the soil solution and thus easily available for plant extraction. But in the case of modality BF0, plants had to create a gradient before being able to access the Ca that was tightly held on the soil's exchange complex. Indeed, after harvest 1, BF0's soil eCa and Ca_{bio} tend to be lower than the baseline, indicating plants dislodged Ca from the soil exchange complex meanwhile modalities that received fertilizer input, tend to present unchanged (F100, BF33) or increased (BF100, BF66) eCa and Ca_{bio}. Plant's ability to access adsorbed nutrients on soil's various components such as biochar, is strongly linked to their root system, itself highly influenced by the crops nutrient status (Brouwer R., 1962; Roy, 2006). Monitoring root growth would therefore be interesting to study in the case of further investigation.

Since for all modalities, eCa represented 75% of the effective CEC, Ca nutrient status could explain why the sole application of biochar resulted in a significantly lower effective CEC than the conventional treatment (appendix E). Like observed for Ca and soil H_2O pH, soil effective CEC presented a decreasing tendency from modalities BF100 to BF0 according to the fertilizer application gradient, with the conventional treatment (F100) presenting a lower mean effective CEC than treatment BF100 (figure 17E). Additionally, BF0 presented a lower pH than other modalities. An increase in acidity meant, there was an increase in the concentration of hydrogen ions (H^+). This could cause protonation of variable charges polymers resulting in a reduced effective CEC, and thus in a reduced capacity to retain other essential cations (Chaplain et al., 2011).

The absence of mineral fertilizer didn't cause a decrease in the above ground mineralomass of any other nutrient. Indeed, SEM chemical qualification revealed detectable amounts of K, P, Ca, Mg, Na, Al at the surface of the charged biochar (figure 21 d and f). Joseph et al. (2018) investigated the mechanisms of nutrient retention in a high temperature wood biochar charged through co-composting (compost feedstock consisted of animal manures, straw, stone meal, soil, and mature compost). Similarly to the results presented in this study, SEM characterization of the composted biochar revealed that its surface coating had a high concentration of C and O and detectable concentrations of P, Al, Mg, Ca. The obtained results in the present study suggest that when nutrient-loaded biochar is put into a low nutrient soil, plants were able to desorb nutrients that were fixed at the charged biochar's surface.

As to co-application, reducing the amount of fertilizer to 33% did not significantly affect any of the analyzed macro and micro-nutrients' (Al, B [5], Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) bioavailability (appendix F) or biomass mineralomass (appendix H) compared to the conventional treatment. Indeed, fertilizer's input in Al, Fe, K, Mg, Mn, Na, P, and Zn [mg] were inferior to insignificant compared to charged biochars' input (appendix I), explaining why a reduction in the applied quantities of mineral fertilizer didn't induce any mineralomass reduction in these nutrients.

As for N, no significant difference was observed for soil N_{tot} (figure 16A). Yet, the colorimetric KCl extraction revealed a significantly lower soil NH_4 content when biochar was applied alone (BF0) but this difference was only significant compared to the conventional treatment (F100) (appendix F). This could be linked to the 0.5-pH unit difference between these two modalities (appendix D). As the pH decreased, the availability of negatively charged sites for $NH_4{}^+$ adsorption also decreased leading to a reduced amount of soil NH_4 (Chaplain et al., 2011).

In accordance with soil NH_4 content, the sole application of charged biochar (BF0) led to a significantly lower biomass N_{tot} content than the conventional treatment (F100) (figure 23). This was also reflected by BF0's NNI (table 12). The latter was bellow 1, indicating that in absence of fertilizer, even though charged biochar is present, BF0's soil columns were in N deficiency. Nevertheless, reducing the amount of fertilizer (BF66, BF33) had no significant effect on the crop's N_{tot}. Indeed, while charged biochar's sole application (BF0) led to significantly lower NNI than all other treatments, in the presence of fertilizer (F100, BF100, BF66, BF33) NNI values were close to 1. This can also be observed on figure 24 placing all samples in spring wheat's N CDC. It is interesting to note that while samples from modalities F100, BF100 and BF66 are located closely around the N critical curve, BF33 and BF0 samples tend to be placed in the lower part of the graph. Similarly, it is worth underlining the clear decreasing tendency according to the application gradient that can be observed on biomass N_{tot} and soil NH_4 graphs between the different modalities (figure 23A and 18A). The fact that F100's mean N_{tot} and NH_4 was higher than that of BF100 in both cases, would suggest that the charged biochar's low N content caused the equilibrium to be in the direction of the nutrients binding to the biochar, thereby reducing the N available for the wheat crops (Kammann et al., 2015).

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[5] B_{bio} and $B_{fertilizer}$ were <DL

Relationship between soil properties, nutrient status, and crop yield

At harvest 1, the sole application of charged biochar (BF0) or reducing the amount of recommended fertilizer (BF66, BF33) didn't result in a significantly lower yield than the conventional treatment (F100) (appendix G). Similarly, at harvest 2, no significant difference in terms of leave and stem yield, grain yield, or number of grains was observed and no influence on the growth rate could be seen (appendix G, figure 26B). All HI were statistically equal (figure 26A), indicating that all modalities converted photosynthetic products (stem and leaves) into harvested products (grains) with the same efficiency despite the different treatments. Since the absence or reduction of fertilizer input didn't have a negative effect on crop yield and characteristics, it can be concluded that none of the modalities experienced a limiting nutritive stress, despite the observed differences in Ca, S and N mineralomass.

Nonetheless, like previously outlined for biomass N_{tot}, a general tendency can be observed on graphs A to D (figure 25); the median yields (A and B), grain yield (C) and number of grains (D) decrease from modality BF100 to BF0 according to the fertilizer gradient. Knowing that in case of optimal solar radiation and water supply conditions, N status becomes the primary factor limiting crop yield, and can thus potentially explain the observed tendencies on yield parameters. Indeed, N deficiencies can potentially lead to reduced photosynthetic rates since the nitrogen status of a cereal crop determines chlorophyll and Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) synthesis, both crucial actors of the photosynthesis, affecting radiation use efficiency of individual leaves and thus, biomass production (Hay, Porter, and Hay, 2006).

Similarly, S is essential for protein production, involved in the formation of chlorophyll and in the activation of enzymes. Plants deficient in S will therefore present retarded growth and delay in cereal maturity. Ca on the other hand, is a part of the architecture of cell walls and membranes. It is involved in cell division, growth, root lengthening and activation or inhibition of enzymes. In case of Ca-deficiency, leaves become small, distorted, cup-shaped, crinkled, and dark green and cease growing (Roy, 2006). In spite of BF0's lower S and Ca mineralomass at harvest 1, the previously described deficiency signs weren't observed, indicating that the sole biochar treatment was still able to answer the crops S and Ca needs. Given the fact that modalities that received 100% of the recommended fertilizer doses along with charged biochar (BF100) tend to present higher mineralomass than the conventional treatment (F100) for most of the studied macro and micro nutrients (appendix 22), would suggest that BF0's missing fertilizer input was compensated by charged biochar. Nevertheless, without control plots this can not be stated with certainty.

Furthermore, even if F100's median yield indicators were lower than modality BF100, addition of charged biochar to 100% of the recommend fertilizer doses didn't significantly increase any of the yields or number of grains (appendix G). Poor responses of crop yield to the application of charged biochar at micro-application rates have been observed before (Abedin, 2018; Schulz, Dunst, and Glaser, 2014). The beneficial effect of biochar on crop yields were explained by the high soil pH increases and liming capacity induced following its application (Alburquerque et al., 2013). Since in the present study, no effect on soil's H_2O and $CaCl_2$ pH could be observed following the addition of charged hard-wood biochar, this could possibly explain why no significant increase could be observed on crop yields.

It is often stated that biochar's effect on soil is linked to long term phenomena like surface oxidation and bio-activation with soil microbes and fungi growing on the biochar surface and can therefore increases over time through (Schulz, Dunst, and Glaser, 2014). Given the limited duration of the present trial, it could be that biochar's beneficial effect simply didn't had the time to manifest.

However, the SEM images (figure 21 g) suggest that the charged biochar used in the present trial was already colonized by microbes. On top of that, since the experimental soil was autoclaved, no micro-organisms were present in the columns except for those contained by the charged biochar. Therefore, if the number of micro-organisms in the charged biochar was too low to ensure its bio-activation, it's effect could never have started, no matter the length of the trial. Clearly, evaluating the true value of this hypothesis demands further investigations on biochar and micro-organisms interactions.

Limits of the study

While experimental conditions were carefully chosen to be as close to field conditions as possible, time, space and budget limitations strongly influenced some important aspects of the trial. Undoubtedly, the use of soil columns filled with autoclaved soil introduced limitations in reproducing the full complexity of soil-plant interactions, introducing biases.

Firstly, the absence of control plots hindered the comparison of the tested treatments with the nutritive status of a crop that grew without any input (no mineral fertilizer or charged biochar). This makes it difficult to say if it was thanks to the charged biochar nutritive input that the crops didn't experience severe nutrient deficiencies or if the soil's original nutrient content was sufficient to assure the crop needs.

Secondly, the nutritive budget analysis was not conducted at the end of the crop cycle, preventing the assessment of grain nutrient content.

Thirdly, although spring wheat is a representative crop in Canada's agricultural landscape and can be grown on almost any soil (Roy, 2006; Statistics Canada, 2022) it may not be the most suitable choice for studying coarse-textured acidic soils in the specific context of British Columbia. Consideration of a species that is often grown on acidic soil like blueberries could have provided a more realistic representation of crop growth and interactions in this region (Hancock and Draper, 1989; Roy, 2006). However, the extended growing cycle of blueberries does not align with the limited duration of a greenhouse trial.

In the same spirit, the short period of experimentation made it impossible to observe the often discussed evolution of biochar effect over time (Burgeon, Victor, 2021). Indeed, even though results didn't show any significant effect of biochar application on crop yields during the present 3-month trial, maybe biochar's slow nutrient release capacities would have been beneficial to future crops.

Finally, it should be noted that all soil columns were flushed with clear water (volume between 500 and 1000 mL) 24 hours prior to harvest 1, in order to analyze potential differences in terms of leaching. The obtained data wasn't presented because it wasn't considered relevant to the objectives of this study. Unfortunately, by doing so, all nutrients that were previously contained in the soil solution were lixiviated. Knowing that 70% of the absorbed N ends up in wheat grains (Roy, 2006) and that their production was only at a very early stage at harvest 1 (figure 12), Z45) the flushing may have disrupted part of the treatment factor's influence on grain yield. Soil columns that were treated with charged biochar, could have beneficiated from charged biochar's known slow nutrient release capacity (Ding et al., 2016), while mineral fertilizer's nutrient input no longer mattered since the applied fertilizer was much likely lixiviated (Zebarth, Paul, and Van Kleeck, 1999).

Contribution

Through this work, the main goal for the student was to design and conduct an experiment to answer the predetermined objectives presented in section 4 of the introduction. The student achieved to:

- Develop a project taking into consideration field, time, and budget constraints
- Plan and conduct an experiment
- Process laboratory measurements and analysis
- Collaborate and take initiatives to accomplish the predetermined objective
- Work in a new environment and adapt to the work dynamic

V Conclusion and perspectives

The production and use of conventional mineral fertilizers comes with multiple environmental issues. But given its role in ensuring global food security, conventional agriculture only tends to intensify fertilizer inputs. Not only does this increase the adverse effects linked to its use, but this also comes at a high cost to farmers. In the context of British Columbia, one potential way of answering the necessity to reduce the amount of applied mineral fertilizers without affecting crop yields, would be to co-apply fertilizer with micro-doses of charged biochar, considering the opportunity for local biochar raw material.

In order to assess the effect reduced fertilizer inputs have on the nutritive status and yield of a spring wheat crop when applied along with micro doses of charged biochar (3t/ha), a 3-month greenhouse trial was conducted on 25 soil columns filled with a coarse textured acidic soil.

Results showed that reducing the amount of fertilizer to 33% did not significantly affect any of the analyzed macro and micro-nutrients' soil bioavailability, biomass concentration or mineralomass compared to the conventional treatment. None of the treatments had a significant effect on crop grain yields. Even so, the sole application of charged biochar significantly reduce soil NH_4, biomass N_{tot} and Ca and S mineralomass. This was explained by the used biochar's content in the respectively considered elements. Given biochar's low alkaline minerals content and the micro-application rate, the often-expected liming effect following its application wasn't observed. This could potentially explain why even when charged biochar was co-applied with 100% of the recommended fertilizer doses, yields didn't increase significantly.

On the hole, considering the present study's findings, reducing the recommended mineral fertilizer requirements of a spring wheat crop without affecting its yield, could be possible when co-applied with micro dosed charged biochar. If the aim becomes to also obtain higher yields, it would be interesting to test higher but still realistic (<10t/ha) charged biochar application rates. Another angle would be, in agreement with BC's available charging feedstock, to test different charging methods in order to obtain biochar with higher alkaline minerals and essential S and N content. Nonetheless, the lack of knowledge on charged biochar's long term nutritive value, make it difficult to predict how this effect evolves over time. In addition, biochar's effect is known to be very site-specific and affected by numerous soil-plant interactions. This underlines the importance to pursue further long-term field investigations before generalizing the present conclusions to field conditions.

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Appendices

A Biochar composition

	Charged biochar
N [%]	0,97 ± 0,04
C [%]	74,64 ± 1,67
S [%]	0,27 ± 0,02
pH H_20 (1:1, soil:water)	10,2 ± 0,1
pH $CaCl_2$ (1:2, soil:$CaCl_2$)	10,1 ± 0,1
eAl [$cmol^+$/kg]	0,2 ± 0
eCa [$cmol^+$/kg]	6,5 ± 0,3
eFe [$cmol^+$/kg]	0,01 ± 0
eK [$cmol^+$/kg]	46,5 ± 5,6
eMg [$cmol^+$/kg]	20,0 ± 1,6
eMn [$cmol^+$/kg]	<DL
eNa [$cmol^+$/kg]	13,1 ± 2,1
Effective CEC	86,3 ± 8,6
Al [mg/kg]	857,3 ± 94,2
Ca [mg/kg]	4792,0 ± 329,6
Cu [mg/kg]	10,3 ± 1,5
Fe [mg/kg]	1340,7 ± 374,9
K [mg/kg]	11300,0 ± 1909,6
Mg [mg/kg]	2240,7 ± 172,5
Mn [mg/kg]	90,0 ± 5,3
Mo [mg/kg]	6,9 ± 3,0
Na [mg/kg]	1494,7 ± 287,4
P [mg/kg]	1132,3 ± 28,0
S [mg/kg]	1069,7 ± 104,8
Zn [mg/kg]	42 ± 4

B Fertilizer composition

	Fertilizer [mg/L]
Al	6
B	<DL
Ca	<DL
Cu	<DL
Fe	8,2
K	84527
Mg	13
Mn	1,5
Na	292
P	16183
S	94506
Si	7
Sr	<DL
Zn	4

C Column building protocol

1. Weight 2644g of dry Totem field soil
2. Poor this mass, in the first 21cm of the column
3. Weight 1531g of dry Totem field soil
4. Weight 11.55g of activated biochar
5. Mix soil and biochar homogeneously
6. Poor the mix in the last 15cm

D Soil H_2O and $CaCl_2$-pH

	Baseline pre-harvest	F100	BF100	BF66	BF33	BF0
H_2O pH	4,3 ± 0,3 a	5,3 ± 0,3 b	5,2 ± 0,2 b	5,1 ± 0,2 b	5,2 ± 0,1 b	4,8 ± 0,1 ab
$CaCl_2$ pH	4 ± 0,2 a	4,5 ± 0,1 bc	4,4 ± 0,2 bc	4,4 ± 0,1 bc	4,5 ± 0,04 c	4,1 ± 0,1 ab

E Soil exchangeable elements and effective CEC [cmol$^+$/kg]

	Baseline pre-harvest	**F100**	**BF100**	**BF66**	**BF33**	**BF0**
eAl	0,3 ± 0,1 c	0,05 ± 0,01 a	0,06 ± 0,01 a	0,06 ± 0,01 ab	0,07 ± 0,01 abc	0,2 ± 0,02 bc
eCa	2,0 ± 0,3 ab	2 ± 0,1 bc	2,4 ± 0,3 c	2,3 ± 0,1 bc	2,1 ± 0,2 abc	1,8 ± 0,2 a
eFe	0,008 ± 0,001 a	0,01 ± 0,001 a	0,01 ± 0,002 a	0,01 ± 0,001 a	0,011 ± 0,002 a	0,011 ± 0,001 a
eK	0,08 ± 0,007 ab	0,3 ± 0,04 bc	0,3 ± 0 c	0,3 ± 0 c	0,2 ± 0,06 abc	0,1 ± 0,01 ab
eMg	0,33 ± 0,07 c	0,29 ± 0,02 ab	0,35 ± 0,04 bc	0,33 ± 0,02 bc	0,32 ± 0,05 ab	0,26 ± 0,02 a
eMn	<DL	0,02 ± 0,01 a	0,02 ± 0,01 a	0,01 ± 0,01 a	0,02 ± 0,01 a	0,01 ± 0,01 a
eNa	<DL	0,04 ± 0,004 a	0,05 ± 0,004 a	0,04 ± 0,005 a	0,05 ± 0,005 a	0,04 ± 0,004 a
effective CEC	2,7 ± 0,3 bc	2,9 ± 0,1 bc	3,2 ± 0,3 c	3,0 ± 0,1 bc	2,7 ± 0,2 ab	2,4 ± 0,2 a

F NH_4, NO_3 and bioavailable nutriment content in soil [mg/kg]

	Baseline	**F100**	**BF100**	**BF66**	**BF33**	**BF0**
NH4+	0,59 ± 0,15 a	52,86 ± 24,85 c	36,60 ± 14,13 bc	29,70 ± 14,72 abc	25,78 ± 6,57 abc	3,54 ± 1,38 ab
NO3-	1,3 ± 0,2 a	0,2 ± 0 a	0,8 ± 0,5 a	0,9 ± 0,8 a	0,6 ± 0 a	0,4 ± 0,2 a
Al	555 ± 155 a	400 ± 37 a	475 ± 82 a	523 ± 67 a	477 ± 63 a	490 ± 53 a
B	1,25 ± 0,129	<DL	<DL	<DL	<DL	<DL
Ca	328 ± 65 ab	332 ± 29 b	366 ± 35 b	361 ± 19 b	312 ± 20 ab	263 ± 27 a
Cu	6,5 ± 2,8 b	2,1 ± 0,2 a	2,9 ± 0,6 ab	3,2 ± 0,5 ab	2,9 ± 0,5 ab	3,00 ± 0,5 ab
Fe	315 ± 33 b	148 ± 10 ab	157 ± 14 ab	154 ± 11 ab	148 ± 12 ab	145 ± 7 a
K	36 ± 6 a	118 ± 17 bc	133 ± 9 c	120 ± 10 bc	95 ± 17 abc	50 ± 9 ab
Mg	58 ± 16 b	33 ± 0 ab	39 ± 5 ab	37 ± 4 ab	35 ± 5 ab	31 ± 3 a
Mn	13 ± 1 a	20 ± 0,8 b	18 ± 2,5 b	20 ± 2,8 b	17 ± 1,3 ab	17 ± 2,51 ab
Na	<DL	9,2 ± 0,84 a	9,6 ± 0,55 a	9,4 ± 1,14 a	9,4 ± 0,89 a	8,6 ± 0,89 a
P	69 ± 28 a	67 ± 6 a	75 ± 8 a	75 ± 10 a	61 ± 6 a	47 ± 2 a
S*	11 ± 0 a	14 ± 3 a	31 ± 10 a	24 ± 11 a	19 ± 7 a	14 ± 4 a
Zn	5 ± 4 a	2 ± 0 a	4 ± 2 a	5 ± 1 a	4 ± 2 a	4 ± 2 a

*During the statistical analysis of bioavailable S in the soil samples, the null hypothesis of the Kruskall Wallis test was rejected but not the nul hypothesis of the Dunn test meaning that the test did not find significant pairwise differences when considering all possible combinations of groups.

G Biomass and grain yield of harvest 1 and 2

	F100	**BF100**	**BF66**	**BF33**	**BF0**
Yield h1 [t/ha]	1,58 ± 0,37 ab	1,82 ± 0,47 b	1,75 ± 0,16 ab	1,56 ± 0,37 ab	1 ± 1 a
Yield h2 [t/ha]	3,16 ± 0,36 a	3,23 ± 0,73 a	2,93 ± 0,82 a	2,63 ± 0,77 a	2 ± 0 a
Grain yield [t/ha]	2,45 ± 0,53 a	2,14 ± 0,70 a	2,03 ± 0,81 a	1,36 ± 0,90 a	1 ± 0 a
Number of grains [column^{-}1]	88,2 ± 23,42 a	78,25 ± 19,86 a	80,8 ± 20,97 a	57,4 ± 24,29 a	60 ± 11 a

H Above ground biomass mineralomass at harvest 1

	F100	BF100	BF66	BF33	BF0
Al	0,10 ± 0,03 a	0,26 ± 0,3 a	0,06 ± 0,03 a	0,08 ± 0,05 a	0,09 ± 0,03 a
B	0,02 ± 0,004 a	0,03 ± 0,004 a	0,02 ± 0,003 a	0,02 ± 0,01 a	0,02 ± 0,003 a
Ca	5,4 ± 0,7 b	4,8 ± 1,1 ab	4,2 ± 0,96 ab	4,1 ± 1,3 ab	3,2 ± 0,6 a
Cu	0,011 ± 0,002 ab	0,014 ± 0,002 b	0,014 ± 0,002 b	0,01 ± 0,003 ab	0,007 ± 0,001 a
Fe	0,23 ± 0,09 a	0,42 ± 0,30 a	0,17 ± 0,06 a	0,14 ± 0,06 a	0,20 ± 0,20 a
K	48 ± 8 ab	63 ± 4 b	57 ± 7 b	50 ± 11 ab	36 ± 8 a
Mg	1,7 ± 0,2 ab	2,0 ± 0,4 b	1,8 ± 0,4 b	1,6 ± 0,3 ab	1,2 ± 0,1 a
Mn	0,79 ± 0,2 a	0,82 ± 0,08 a	0,75 ± 0,09 a	0,69 ± 0,2 a	0,54 ± 0,2 a
Mo	<DL	0,0007 ± 0,0008 a	0,0005 ± 0,0007 a	0,0004 ± 0,0005 a	0,0006 ± 0,0005 a
Na	0,083 ± 0,02 a	0,13 ± 0,05 a	0,081 ± 0,01 a	0,094 ± 0,03 a	0,082 ± 0,03 a
P	4,5 ± 1 a	5,4 ± 1 a	4,6 ± 1 a	3,9 ± 1 a	2,7 ± 1 a
S	7,9 ± 0,7 b	8,7 ± 1 b	6,9 ± 2 b	5,6 ± 2 b	3,8 ± 1 a
Zn	0,064 ± 0,01 a	0,12 ± 0,03 b	0,12 ± 0,03b	0,088 ± 0,03 ab	0,065 ± 0,007 a

I Nutritive input per modality

	F100 [mg]	BF100 [mg]	BF66 [mg]	BF33 [mg]	BF0 [mg]
Al	0,0	9,9	9,9	9,9	9,9
B	<DL	0,1	0,1	0,1	0,1
Ca	<DL	55,3	55,3	55,3	55,3
Cu	<DL	0,1	0,1	0,1	0,1
Fe	0,0>0	15,5	15,5	15,5	15,5
K	47,1	177,6	161,9	146,2	130,5
Mg	0,0>0	25,9	25,9	25,9	25,9
Mn	0,0>0	1,0	1,0	1,0	1,0
Na	0,2	17,4	17,4	17,3	17,3
P	9,0	22,1	19,1	16,1	13,1
S	52,6	65,0	47,5	29,9	12,4
Zn	0,0>0	0,5	0,5	0,5	0,5