INSTITUTO POLITÉCNICO
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NITROGEN DYNAMICS AFTER APPLYING BIOCHAR, ZEOLITES AND MINERAL FERTILIZERS IN SOIL-PLANT ENVIRONMENT OF CORN (*Zea mays*)

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Dissertation presented to the School of Agriculture of Polytechnic Institute of Bragança to obtain the Master's Degree in Agroecology under the double diploma with Federal University of Technology - Paraná.

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Bragança, 2019

To my mom, my reason to live.

Everything I do is because and for you.

AKNOWLEDGEMENT

First, I thank Polytechnic Institute of Bragança and Federal University of Technology - Paraná to give me the opportunity to participate on this double diploma program.

My Portuguese advisors, professor Margarida Arrobas PhD and professor Manuel Ângelo PhD, for all the patience, knowledge, help and incentive throughout every step of this work, even in the hardest times. Thank you for everything, from the field work, until the very end of the writing process. I am very grateful to accept this challenge and to have the chance to work with you.

My Brazilian advisor, professor Laércio Sartor PhD, thank you for being in my academic life, teaching and helping me in almost all the hardest steps.

A special thanks to the IPB's Soil and Plant Laboratory technicians Rita and Ana, you made me learn more than I thought I could. To Soraia, Sandra and David, for all the help, advisory and companionship through the laboratorial processes.

To all my friends in Brazil, whom even by long distance, helped me carry on through all the difficulties and to all my friends in Portugal that gave me strength, support and helped me keep my feet on the ground throughout this process.

The biggest thanks to my family. I would be nothing without you and everything I do is for you. No words could ever measure my love and gratefulness for you.

To all the ones I lost during this process. I hold a little part of you with me every day.

ABSTRACT

Every crop needs a well-nourished soil and balanced nutrition to have a good development. Nitrogen (N) balance is essential for plant development, and it depends on soil physical, chemical and biological properties. Some soil conditioners may have a relevant role in soil available nitrogen. The present work intends to evaluate the effect of soil conditioners such as Biochar, Zeolites and Mineral fertilizers on soil properties, particularly on nitrogen dynamics, and corn crop growth and yield. It is also the objective of this work to evaluate the performance of plants through the determination of chlorophyll fluorescence, green color intensity, nutritional status and production components in field and pot experiments. The field experiment was arranged as a factorial design with three soil conditioners (Biochar, Zeolites and Mineral fertilizers) and four N rates (0, 50, 100, 200 kg N ha^{-1}). The pot experiment was also arranged in a similar factorial design with only two nitrogen rates (0 and 2 g N plant^{-1}). In both experiments there were field sampling, *in situ* measurements, and laboratory analysis, to determine macro and micronutrients in plant tissues when exposed to different soil conditioners. Soil analyses were made only in the field experiment to verify soil properties during the growing season and after harvest. Soil nitrate and ammonium concentration were determined along with the hydrolysable nitrogen and plant apparent nitrogen recovery. For both, field and pot experiment, the use of soil conditioners demonstrated better results in the soil-plant environment. In the field experiment, the Zeolites' treatments showed an aboveground biomass increase, as well as a better nutritional status. Biochar presented higher apparent nitrogen recovery. Soil properties at harvest demonstrate Biochar efficiency and nutritional improvement during the growing season. The different nitrogen rates influenced on soil properties as well. In the pot experiment, the Zeolites + N treatment was the one with better plant nutritional improvement and growth and Biochar + N treatment demonstrated a significant increase in the aboveground biomass.

Keywords: Soil conditioners; nitrogen fertilization; pre-sidedress soil nitrate test, SPAD-readings; NDVI; stalk nitrate test; corn yield

RESUMO

Toda cultura precisa de solo bem provido de nutrientes para seu desenvolvimento. O nitrogênio (N) é essencial para o desenvolvimento das plantas e a sua disponibilidade depende das propriedades físicas, químicas e biológicas do solo. Alguns condicionadores de solo podem ter um papel relevante no nitrogênio disponível no solo. O presente trabalho pretende avaliar o efeito de condicionadores como Biochar, Zeólitos e Fertilizantes minerais nas propriedades do solo, particularmente na dinâmica do nitrogênio, e no crescimento e produtividade da cultura do milho. É também objetivo deste trabalho avaliar o desempenho de plantas através da determinação da fluorescência da clorofila, intensidade de cor verde, estado nutricional e componentes de produção em experimentos em campo e em vaso. O experimento de campo foi organizado em esquema fatorial com três condicionadores de solo (Biochar, Zeólitos e Fertilizantes minerais) e quatro doses de N (0, 50, 100, 200 kg N ha^{-1}). O experimento de vasos também foi organizado em um esquema fatorial similar com apenas duas doses de N (0 e 2 g N plant^{-1}) . Em ambos os experimentos foram realizadas coletas em campo, medições *in situ*, e análises laboratoriais, para observar macro e micronutrientes nos tecidos vegetais quando expostos a diferentes condicionadores do solo. A análise do solo foi feita apenas no experimento de campo para verificar as propriedades deste durante o crescimento e após a colheita. Determinou-se as concentrações de nitrato e de amônio no solo, juntamente com o nitrogênio hidrolisável e nitrogênio aparentemente recuperado. Para ambos experimentos, o uso dos condicionadores demonstrou melhores resultados no ambiente solo-planta. No experimento de campo, houve um aumento de biomassa dos tratamentos com Zeólitos, bem como um melhor estado nutricional das plantas. As plantas com Biochar apresentaram maior recuperação aparente de nitrogênio. As propriedades do solo na colheita demonstram a eficiência do Biochar e a melhora nutricional durante o ciclo da cultura. As diferentes taxas de nitrogênio influenciaram as propriedades do solo. No experimento de vasos, o tratamento com Zeólitos + N foi o que obteve melhor estado nutricional e crescimento de plantas e o tratamento com Biochar + N demonstrou um aumento significativo na biomassa.

Palavras-chave: Condicionadores de solo; Fertilizantes nitrogenados; *Pre-Sidedress Soil Nitrate Test*; leituras de SPAD; NDVI; S*talk Nitrate Test*; Produção de milho.

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ABREVIATIONS LIST

Al	Aluminium
Al_2O_3	Aluminium Oxide
ANR	Apparent Nitrogen Recovery
B	Boron
$B(OH)_3$	Boric Acid
BD	Bulk Density
C	Conditionerrs
Ca	Calcium
$CaCl_2$	Calcium Chloride
CaO	Calcium Oxide
CEC	Cation Exchange Capacity
CO_2	Carbon Dioxide
Cu	Copper
EA	Exchange Acidity
F_0	Minimum Fluorescence
Fe	Iron
Fe_2O_3	Iron (III) Oxide
F_m	Maximum Fluorescence
F_v	Variable Fluorescence
H^+	Hydrogen ion
H_2O	Water
K	Potassium
K_2O	Potassium Oxide
KCl	Potassium Chloride
MAPA	Ministry of Agriculture, Livestock and Food Supply
Mg	Magnesium
MgO	Magnesium oxide
Mn	Manganese
N	Nitrogen Element
N_2	Nitrogen Gas
Na^+	Sodium
Na_2O	Sodium Oxide
NAf	Nutrient Applied as a Fertilizer
NaOH	Sodium Hydroxide
NDVI	Normalized Difference Vegetation Index
NEf	Nutrient Recovery in the Fertilized Plants
NEt	Nutrient Recovery in the Control Plants
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrites
NO_3^-	Nitrate
OH^-	Hydroxyl
OM	Organic Matter

P	Phosphorus
P_2O_5	Phosphorus Oxide
POPs	Persistent Organic Pollutants
PPE	Personal Protective Equipment
PSNT	Pre-Sidedress Nitrate Test
R	Rate
SiO_2	Silicom Dioxide
SPAD	Soil Plant Analysis Development
TiO_2	Titanium Dioxide
TMC	Total Micologic Content
UAN	Urea - Ammonium Nitrate
Zn	Zinc

1. INTRODUCTION

1.1. MOTIVATION AND FRAMEWORK

For the past years, corn (*Zea mays* L.) crops have undergone important technological changes, resulting in significant increases in productivity and production. Among these technologies, it is important to stimulate producer's awareness and the need to improve soil quality, aiming at sustainable production [1]. This improvement in soil quality is generally related to adequate management, which includes, among other practices, crop rotation, no-tillage, fertility management through liming, silage and balanced fertilization with macro and micronutrients, using chemical and/or organic fertilizers [2].

To reach a rational soil fertility management, it is essential to use a series of diagnostic tools for possible nutritional problems that, once corrected, will increase the chances of success in agriculture [1]. Among the essential elements for plant growth, nitrogen should be highlighted, as it is the most expensive and required in larger quantities by most crops, especially corn [3].

Nitrogen in high concentrations is harmful for soil, water and the atmosphere. Anthropogenic activities have part in this problem's recent increase. Although nitrogen is an essential element for living beings and for a good functioning of the ecosystem, in excess it can become a pollutant factor, changing the ecological balance [4].

For plants, nitrogen is an essential nutrient in their development, and its availability in cropping systems is sometimes limited. For this reason, the addition of nitrogen as fertilizer in agriculture is increasingly common and often unavoidable [5]. In many cases, neither the requirements of different crops nor the availability of this nutrient in the soil are known, which can lead to high amounts of nitrogen fertilizers to the soil applications, in order to guarantee high yields [6].

The excessive application of nitrogen dosages, coupled with its high mobility in the soil-plant-atmosphere system, can translate into economic damages and have important implications in human and animal health, and the environment. The economic losses result from the fact that the production value does not increase proportionally to the nitrogen applied as fertilizer, reaching a point in which it is commonly referred to as "economic optimum", where possible increases in production do not cover expenditure on the excess fertilizer applied [7]. In the nitrogen applied as fertilizer, the fraction that is not recovered by the plants constitutes an economic loss, since nitrogen is among the most expensive macronutrients [8].

In human health, the implications of the excessive nitrogen dosages use are related to the presence of nitrates in excessive amounts in drinking water (due to leaching losses) and in plants (due to the ease in which they absorb nitrogen in addition to their immediate metabolic needs and accumulate it in their tissues in the form of nitrates). In animal health, high levels of nitrates in fodder can lead to toxicity, especially in ruminants. Other antinutritive compounds, such as amides and oxalic acid, may also accumulate in fodder when nitrogen is available in excessive amounts [9].

Recently researches have tried to better study soil conditioners in order to improve their physico-chemical characteristics and increase agricultural crops yields through better control of nutrient availability [10].

Among the conditioners that can be used in the nitrogen dynamics, biochar and zeolites have been highlighted. Biochar, in its origin, was only used for agriculture as a way of helping in various crops production. Over time, however, it has been more studied and it a tendency that these studies expand every day, always aiming soil properties improvement for crop production. Biochar is a soil conditioner that results from the pyrolyzed biomass from several sources in the absence of oxygen and is capable of mitigating impacts in the area of water, effluent and soil recovery, as well as being a great store of soil carbon dioxide [11].

The zeolites are a group with more than 80 naturally occurring minerals, which have a three-dimensional structure with interconnected cavities that confer property and advantageous characteristics, standing out its use in the development of slow release fertilizers [12].

Mineral fertilizers are derived from ores. They are also known as synthetic because they undergo a breaking process in order to separate the ores from the impurities. They can be made from petroleum products, rocks and even from organic sources. The nutrients are available immediately to the plant, so they are absorbed quickly and therefore require care since they can even cause problems [12].

The main purpose of this work, it to understand how biochar, zeolites and mineral fertilizers can assist in controlling nitrogen dynamics in the soil-plant environment in corn crops.

1.2. OBJECTIVES

The present work intends to evaluate the effect of soil conditioners such as biochar, zeolites and mineral fertilizers on soil properties, particularly on nitrogen dynamics, and corn crop growth and yield.

It is also the objective of this work to evaluate the performance of plants through the determination of chlorophyll fluorescence, green color intensity, nutritional status and production components.

1.3. DOCUMENT STRUCTURE

This dissertation is organized in six chapters. In this Chapter 1 the theme of the research and the motivation is described, the intended objectives, and finally the document structure is presented.

In Chapter 2 the bibliographic review is presented, exposing some of the main studies related to the topic of interest and a synthesis of the knowledge regarding the general characteristics of corn crops, nitrogen cycle, plant and soil nitrogen use, nitrogen management in agroecosystems and nitrogen-based fertilizers, and lastly the soil conditioners utilized in this study.

Chapter 3 focuses on the work plan developed to achieve the objectives set out in Chapter 1. The study site and the methodology applied for field sampling, *in situ* measurements and laboratory analysis are described.

Chapter 4 is dedicated to present the results related to Chapter 3, after data analysis. Chapter 5 intends to discuss the results found on Chapter 4 and compare them to previous work in order to see if there were positive and significant results.

Chapter 6 aims to systematize the knowledge generated during the research, its conclusions and suggestions for future work.

2. BIBLIOGRAPHIC REVIEW

2.1. CORN CROP

Corn *(Zea mays* L.) is a grass of the Poaceae family. It is an annual crop, cultivated mostly during the summer. Corn plants may produce more than one stalk from each seed. It is a species of erect habit and low tillering. Corn is a monoic species with male and female inflorescences appearing in different parts of the plant. Corn plants are classified in the C_4 plants group, with wide adaptation to different environmental conditions [13].

To express its maximum productive potential, as a C_4 species, the crop requires high temperature, around 24 and 30 °C, high solar radiation and adequate water availability [14].

Male spikelets are gathered in terminal verticil spikes. The corn grain is a caryopsis, in which the pericarp is fused to the tegument of the seed itself. The female spikelets are welded on a common axis in which several rachides are gathered protected by bracts. The female flower has a single stigma [15].

The corn grain is mainly used for human and animal consumption, being an essentially energetic food, since its main component is starch. The protein content normally found in the grain is in the range of 9 to 11% [13]. In addition to presenting low protein content, the quality of the protein is lower than that found in other cereals, since most of it consists of zein, which is poor in amino acids, such as lysine and tryptophan [15].

Corn's adaptability allows its cultivation in practically all regions of the globe presenting a warm season [16]. This is due to the evolution of the species and also to the genetic improvement of corn varieties.

2.1.1. History and evolution

The history of corn has its beginning about 10,000 years ago, with agriculture's emergence. Central America, more specifically where Mexico is located, was the cradle of corn plants' domestication, through artificial selection and/or selective seed breeding [17].

According to Doebley [18], the ancestry of corn remained a mystery for many decades, but after several studies, it was proven that, despite the enormous morphological difference, the genetic similarities of the species with a plant called teosinte were undeniable, having the same number of chromosomes and extremely similar gene arrangements. Indeed, up to the present, crosses between the two species are being made, creating hybrids that can reproduce naturally.

Over time, corn cobs became larger, with more rows and grains, eventually taking the form of modern corn [19].

2.1.2. Production

Corn's evolutional process has made its production grow worldwide. The development of corn production and marketing should be analyzed, preferably, from the perspective of production chains or agroindustry systems, as corn is raw material for a hundred different products, but approximately 70% of the corn produced in the world is consumed in the pig and poultry production chains [20].

According to Guth [21], in the 2017/18 harvest corn production ran around 1,02 billion tonnes. The United States of America dominates world corn production, accounting for about 37 percent of the planet's total grain yield. The country produced approximately 360 million tonnes of corn in the 2017/18 crop [22]. China is the world's second largest producer, with 215 million tonnes of corn produced. Brazil comes in third place in the largest producers with 95 million tonnes [23]. The countries of the European Union and Argentina close the list of the largest producers of the grain, with 6% and 4% of the total, respectively [22].

When it comes, specifically, to the European Union, France is the largest corn producer, with 11 941 thousand tonnes and Portugal comes in fourteenth place, with around 780 thousand tonnes [24].

In Portugal, the cereals sector is experiencing one of its most serious crises, as a result of the low prices paid for the grain. However, the strong dynamics and vitality that characterizes the corn sector in Portugal has led to an increase in sown area [25]. In fact, according to provisional data from Anpromis [26], corn cultivation currently occupies an area of 118 220 hectares. Compared to previous years, the trend of decline in the last three marketing years has been reversed, with an increase of 2 555 hectares compared to 2017.

According to Anpromis [26] this increase is due to two key factors: reducing the area of certain irrigated arable crops and increasing the value of domestic grain production, which has resulted in an increased demand from the agri-food industry.

Portugal is historically dependent on corn imports, but if the levels of self-provisioning were 45% in 1989, the decrease in production and the increase in consumption led to a particularly low current value (about 31%) [27].

This trend of declining production in cereals in general and corn has prompted the government to set up the Grains Working Group to propose a national strategy for the promotion of cereal production [25].

In Portugal, corn crop production is closely linked to irrigation, taking advantage of the soil-climatic potential that the geographical positioning provides, but also because this location makes irrigation an essential contribution to the vegetative development of the crop [14].

Besides irrigation, well-nourished soil and plants are extremely important to keep a stable production, therefore a series of analysis and procedures must be done in order to better produce the demanded corn for each crop cycle [28].

2.1.3. Nutrition and fertilization

The nutritional needs of any plant are determined by the amount of nutrients it uptakes during its growing cycle. The total extraction will, therefore, depend on the yield (total biomass) obtained and nutrient concentration on grains and haulm. Thus, both grain and silage production will have to be available on soil the total amount of nutrients the plant extracts, which must be supplied by fertilization [29].

Mean data from experiments conducted by Coelho [1], with moderate to high fertilizer rates, give an idea of the nutrient extraction by corn grown for grain production and silage. It is observed that the extraction of nitrogen, phosphorus, potassium, calcium and magnesium increases linearly with the increase in production, and that the highest requirement of corn refers to nitrogen and potassium, followed by calcium, magnesium and phosphorus.

In corn, nutrients have different rates of translocation between tissues (stalks, leaves and grains). Regarding the removal of nutrients, phosphorus is almost all translocated to grains (77-86%), followed by nitrogen (70-77%), sulfur (60%), magnesium (47-69%), potassium (26 to 43%) and calcium (3 to 7%) [1]. This implies that crop residues are incorporated into the soil, returning most of the nutrients found in the plant, but mainly potassium and calcium present in haulm.

According to Broch [30], when corn is harvested for silage, in addition to the grains, the vegetative part is also removed, resulting in high extraction and exportation of nutrients. Thus, soil fertility problems will manifest earlier in silage production than in grain production, especially if the former is obtained from the same area for several consecutive years and if a suitable soil management and fertilization system is not adopted. A liming and fertilization program, aiming at the maintenance of high yields, requires a periodic monitoring of soil fertility indexes, through chemical analysis in order to avoid the impoverishment and imbalance of the soil [29].

Once the need for fertilizer application in corn crops has been defined, the next step, and of great importance in fertilization management, aiming at maximum efficiency, is the absorption and accumulation of nutrients in the different stages of development of the plant knowledge, identifying the times when the elements are required in larger quantities. This information, coupled with the potential for losses due to nutrients leach in different soil types, are important factors to consider when applying fertilizers to the crop, mainly under irrigated conditions [1].

For nitrogen and phosphorus, corn presents two periods of maximum absorption during the phases of vegetative and reproductive development or spike formation, and lower rates of absorption in the period between the emission of the tassel and the beginning of the corn cob formation [31].

The results obtained by Cruz [32] on the nitrogen splitting in corn crops show that the non-supply of this nutrient during the initial stage of vegetative development, with an application of the full bloom rate (65 BD), as well as excessive number of applications on the experiments, presented less efficiency than the application at the time of planting and in the stage of vegetative development.

It is important to know the nutrient demands and to follow an adequate fertilizer management program during the growing cycle, contributing to a higher fertilization efficiency [1]. However, for many farmers, it is easier to apply the fertilizers into irrigation water, directing the parcel, mainly of the potassic and nitrogen fertilizations, sometimes in excessive number, without considering the crop requirement when related to the absorption curve and the potential of nutrient losses due to their mobility in the different types of soil [29].

2.2. NITROGEN

Nitrogen is a chemical element represented by the symbol N, its atomic number is 7 and it has an atomic mass of 14.00674 u (7 protons and 7 neutrons), represented in the group (or family) 15 of the periodic table [33].

Nitrogen was discovered by the Scottish physician Daniel Rutherford in 1772, as a separable component of air [34]. Under normal conditions it forms a diatomic, colorless, odorless, tasteless and mainly inert gas, which constitutes 78.08% of the volume of atmospheric air [35]. Although nitrogen within soil and terrestrial vegetation is widely considered to be from the atmosphere, weathered rocks contribute 6 to 17 percent of the total terrestrial nitrogen supply, or 11 to 18 tera-grams of nitrogen annually [36].

Nitrogen is the element that plants need in larger quantities. It is a primary or noble macronutrient. However, due to the multiplicity of chemical and biological reactions, the dependence of environmental conditions and their effect on crop yields, nitrogen is also the element that presents the greatest challenges of handling in agricultural production even in technically oriented properties [5].

2.2.1. Nitrogen cycle

Nitrogen can undergo several transformations in the soil. These transformations are usually grouped into a system called the nitrogen cycle, which can be presented in various degrees of complexity. The nitrogen cycle is appropriate for understanding the management of nutrients and fertilizers. Since microorganisms account for most of these processes, they occur slowly under temperatures below 10 °C. But their rates grow rapidly as the soil becomes warmer [37]. A schematic example of this cycle can be observed in Figure 1.

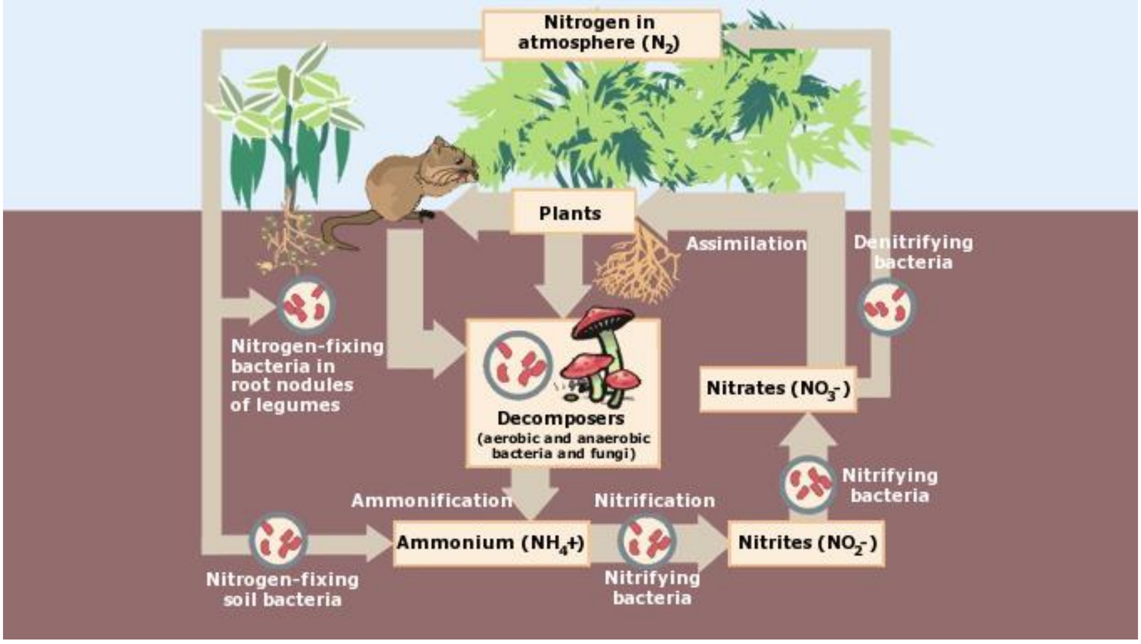


Figure 1. Nitrogen cycle showing the processes that influence on plant and soil development [38].

The center of the nitrogen cycle is the conversion of inorganic nitrogen to organic nitrogen, and vice versa. As the microorganisms grow, they remove NH_4^+ and NO_3^- from the inorganic reserves of the available nitrogen in the soil, converting it into organic nitrogen within a process called immobilization [37]. When these organisms die and are decomposed by others, NH_4^+ excess can be released back into the inorganic reservoir, within a process called mineralization. Nitrogen can also be mineralized when the microorganisms decompose materials that contain more nitrogen than they can use at one time, materials such as legume residues and manure [39]. Immobilization and mineralization are driven by most microorganisms and are even faster when the soil is heated and moisten but not saturated with water. The amount of inorganic nitrogen available for harvesting usually depends on the amount of mineralization and the balance between mineralization and immobilization [40].

Ammonium ions (NH_4^+) not immobilized or rapidly absorbed by larger plants are usually converted rapidly into NO_3^- ions, in a process called nitrification. This is a two-step process, during which the bacteria called *Nitrosomonas* convert NH_4^+ into nitrite

(NO_2^-), and then another genus of bacteria, *Nitrobacter*, converts NO_2^- into NO_3^-. This process requires an aerated soil and occurs so fast that NO_3^- is normally found in bigger proportions than NH_4^+ in soils during the growing season [41].

The nitrogen cycle has several routes through which the nitrogen available to the plants can be removed from the soil. Nitrogen-nitrate is usually more subjected to withdrawal than nitrogen-ammonium. Significant removal mechanisms include leaching, denitrification, volatilization, and crop removal [42].

The nitrate form of nitrogen is so soluble that it leaches easily when excess water passes through the soil. This may be a major mechanism of loss in sooty bruised soils through which water flows freely [43]. But it is a minor problem in soils with a more refined and impermeable texture, in which the percolation is very slow [37].

Younger soils tend to become more easily saturated, and when microorganisms exhaust the oxygen-free supply in moist soils, some of them begin to get it by the decomposition of NO_3-. In this process, called denitrification, NO_3^- is converted into nitrogen gaseous oxides and into N_2 gas, both unavailable to plants. Denitrification can cause large nitrogen losses when the soils are heated and remain saturated for more than just a few days [44].

NH_4^+-nitrogen losses are less common and occur mainly through volatilization. The ammonium ions are basically anhydrous ammonia (NH_3) molecules with an additional hydrogen ion (H^+) added to it [42]. When this additional H^+ is removed from the NH_4 ion by another ion such as hydroxyl (OH^-), the resulting NH_3 molecule can evaporate and volatilize from the soil. This mechanism is even more important in soils with high pH and with large amounts of OH^- ion [45].

Crop removals represents a loss, because the nitrogen in the harvested crop parts is completely removed from the field. The nitrogen in the crop residues is recycled back into the system, and is better still, as immobilized than as removed. Much of it is mineralized and can be reused by the next crop [37].

2.2.2. Nitrogen in plants

Nitrogen has a fundamental role in plant metabolism because it participates directly in the biosynthesis of proteins and chlorophylls [46]. However, it is found in insufficient amounts in most soils, making it essential to exogenous supplies in an adequate concentration to guarantee the growth, development and productivity of corn plants [47]. With this knowledge, losses are minimized and the efficiency of nitrogen use is increased [48].

In corn plants there is an intense nitrogen absorption in the early stages of development, being its deficiency one of the major limitations to productivity. The processes knowledge involved in the incorporation and transformation of the nitrogen in the soil-plant-atmosphere system is essential to management strategies development that can increase its utilization by crops. In the case of corn, if the crop received the application, its absorption rarely exceeds 50% of the applied as mineral fertilizer [49]. This happens because nitrogen applied to the soil is subjected to losses due to leaching, surface runoff, denitrification, ammonia volatilization and immobilization in microbial biomass [50].

Thus, the increase in grain yield depends, among other factors, on the efficiency of nitrogen uptaken and its translocation to growing grains, where the reserve formation compounds will occur. Researches to evaluate the mineral nitrogen obtained from nitrogen-based fertilizers supplied by roots have been done, in order to consider their low efficiency [51]. Foliar nitrogen supplementation is a convenient and rapid practice, used to improve mineral responses and, consequently, plant growth and to correct nutritional disorders at crop stages where soil application becomes inefficient, given the absorption and response [52].

When there is not enough nitrogen in the plant, the symptoms are quite characteristic [53]:

- A slight deficiency of nitrogen will cause a restriction on plant growth, but generally, if it is subtler, it can only be perceived when compared with plants that have an adequate nitrogen supplement [12].
- Moderate deficiencies of nitrogen cause changes in leaf color to light green or yellowish.
- Severe symptoms include necrosis (tissue death), beginning at the tips of older leaves, with the development of V-shaped patterns by the central vein toward the base of the leaf [12].

2.2.3. Nitrogen in soils

Nitrogen in soils exists in three general forms: organic nitrogen compounds, ammonium ions (NH_4^+) and nitrate ions (NO_3^-) [36].

It is known that 95% to 99% of the nitrogen potentially available in the soil is in organic forms, either in plant or animal residues, in a relatively stable soil organic matter or in soil living organisms, mainly microbes, such as bacteria [53]. Organic nitrogen is not directly available to plants, but part of it can be converted to available forms through microorganisms. A very small portion of organic nitrogen may exist in soluble organic compounds, such as urea, which may be slightly available to vegetables [54].

Most of the nitrogen available to plants is in their inorganic forms NH_4^+ and NO_3- (sometimes called mineral nitrogen). The ammonium ions can be bonded to the negatively charged cation exchange complex (CEC) and behave similarly to several other cations in the soil. Nitrate ions do not bind to soil solids because they carry negative charges, but exist dissolved in soil water, or precipitated as soluble salts under dry conditions [55].

Nitrogen in the soil, which can eventually be used by plants, comes from two main sources: nitrogen-containing minerals and the vast amount of nitrogen in the atmosphere [36]. The nitrogen found in soil minerals is released with their decomposition. This process is generally somewhat slow and contributes little to nitrogen nutrition in most soils. In soils containing large amounts of enriched NH_4^+ argils (both natural or resulting from the addition of NH_4^+ as a fertilizer), nitrogen provided by the mineral fraction may be significant during several years [56].

Atmospheric nitrogen is a substantial nitrogen source in soils. In the atmosphere, it occurs in the rather inert form of N_2 and needs to be converted before it becomes useful in the soil. The amount of nitrogen added to the soil is thus directly related to the electrical activity of the atmosphere [37].

Bacteria such as *Rhizobia*, that infect roots and receive nutritional energy from leguminous and other nitrogen-fixing plants, can attach much more nitrogen per year. Therefore, plants that have a good nodulation usually don’t respond well to nitrogen-based fertilizer, because the nitrogen needed in their development is already supplied by the *Rhizobia* bacteria [40].

2.3. AGROECOSYSTEMS

An agroecosystem is classified as any type of ecosystem modified and managed by human beings with the objective of obtaining food, fibers and other materials of biotic origin [57]. The concept includes the typical example of traditional agriculture, including its ecological and organic versions, which can be characterized by its adaptation and adjustment to the possibilities offered by nature, trying to maintain its basic processes, as conventional and industrial agriculture, in which the dominant objective is associated with maximizing profitability. It also includes extensive livestock systems, with or without the presence of trees. Many of them maintain mixed uses and can be classified as agrosilvopastoral [58].

Considering current agriculture’s objectives, the supply services provided by an agroecosystem will prevail. But the ways that traditional agricultural management was guided, shows its effects among us until now and can be observed in many current agricultural landscapes. This management obtains products in a stable manner, in a self - sufficient way and reduces possibilities for possible external inputs that could lead to negative results. Therefore, agroecosystems are arising as incorporated structural elements and processes that help maintain a certain level of ecological integrity, reinforcing their capacity to deliver services according to their demand[59].

The biggest difference between agroecosystems and ecosystems is their strict dependence on human management to ensure the functioning of essential ecological processes (productivity, fertility recovery, water cycle, herbivory, soil), that gives them their own, very original characteristics (agrobiodiversity, cultural regulation, infrastructures, agrarian landscape) [60].

Agroecosystems are for this reason enriched with numerous cultural elements, as domestication, controlled and adapted management of plants and animals, constituting original landscapes that respond under different conditions to specific purposes of production [61].

The agroecosystem’s fertility depends fundamentally on photosynthesis and the production of plants phytomass, since the plants are responsible for the energy input into the system, thus feeding all forms of life in the agroecosystem [62]. Plants are the main sources of organic matter in the soil, whose presence may constitute an indicator of soil quality. The amount of plant biomass assigned to the agricultural system depends on the type of crop cultivation, management employed and the production system [63].

In the soil-plant system, the nutrients are in a state of constant transfer, because the plants absorb it and use them in metabolic processes, returning them to the soil at the end of the cycle. In addition to nutrient cycling in the agroecosystem, there are other nutrients inputs and outputs, either through the addition of organic and inorganic fertilizers, rock weathering processes, biological fixation and erosion, leaching, harvesting, volatilization and fire use [64].

2.3.1. Nitrogen management in agroecosystems

An ideal farm model presupposes the correct use of land without soil and other natural resources degradation, through the combination of agro-ecological and socio-economic planning. The recycling of nutrients and the use of crop residues as a source of organic material are relevant for the proper management of soil fertility [63].

The efficiency of nitrogen management plants depends on the understanding of nitrogen cycles in agroecosystems, avoiding to the maximum the losses of this element in the productive process [65].

As strategies to reduce potential nitrogen losses in agricultural systems and to mitigate greenhouse gas emissions, it is possible to adopt cover crops under no-tillage system, organic production practices and nitrification inhibitors during fertilization. The latter delay the conversion of ammoniacal nitrogen to nitrate, and potentially result in lower nitrogen losses due to leaching and nitrous oxide emissions. On the other hand, the use of legumes as soil cover reduces the need for nitrogen fertilizer application, reducing losses due to leaching, ammonia volatilization and greenhouse gas emissions [37].

2.3.1.1. Nitrogen based fertilizers application

Application decisions should maximize the availability of nitrogen to crops and minimize potential losses [66]. The roots of a plant usually will not grow along the root zone of another plant. Thus, nitrogen must be positioned where all plants have access to it. Broad applications serve this purpose. The bandage also gives account when all the streets are next to a band. For corn, the bandage of anhydrous ammonia or urea -

ammonium nitrate (UAN) in the center of alternating streets is usually as efficient when wrapping in each center because all the streets have access to the fertilizer [67].

Soil moisture conditions are required for nutrient uptake. Application below the soil surface can increase the nitrogen availability under dry conditions, because the roots are more likely to find nitrogen in moist soils with this application [52]. Injection of UAN coverage can lead to higher corn productivity than superficial application over the years when dry weather succeeds in coverage. In the years when the rainy season begins shortly after application, application under the surface is not so critical [67].

Application under the surface is usually used to control nitrogen losses. Anhydrous ammonia can be positioned and sealed below the surface to eliminate losses by direct volatilization of the ammonia gases [53]. The volatilization of urea and UAN solutions can be controlled by incorporation or injection. The incorporation of urea materials (mechanically or through brief rainfall after application) is especially important in no-till situations in which volatilization is aggravated by large amounts of organic material on the soil surface. The application of small amounts of nitrogen as UAN in herbicidal sprays is, however, of little concern [67].

The main mechanisms of nitrogen fertilizers loss are denitrification, leaching and volatilization. Denitrification and leaching occur in soils under very humid conditions, while volatilization is more common when soils are less moist and drying [45].

Using an NH_4^+ nitrogen source to acidify the soil because the hydrogen (H^+) ions, released during nitrification of NH_4^+, are the main cause of soil acidity. Over time, acidification and soil pH reduction can become significant [55].

Nitrogen fertilizers containing NO_3^- but no NH_4^+ make the soil slightly less acidic over time but are generally used in much smaller quantities than in others. Acidification due to NH_4^+ nitrogen is a significant factor in the acidification of agricultural fields but can easily be controlled by common liming practices [37].

2.3.1.2. Nitrogen application time

The timing of application has a great effect on the efficiency of nitrogen management systems. Nitrogen should be applied in such a way as to avoid periods of significant loss and to provide adequate nitrogen when the crop needs it most. Corn absorbs most of the nitrogen by mid-summer [66].

Thus, widespread availability at these times is critical. If losses are expected to be minimal, or can be effectively controlled, applications before or immediately after planting are efficient for the crop. If significant losses, especially those subject to denitrification or leaching, are anticipated, split applications, in which much of the nitrogen is applied after the emergence of the crop, can be efficient in reducing losses [68].

Autumn applications for corn can be used on well-drained soils, particularly if nitrogen is applied as an anhydrous ammonia, however, fall applications should be avoided on poorly drained soils because of the near-impossible potential for significant denitrification losses [69].

Most of the nitrogen supplied to a crop will be applied after substantial planting growth, or positioned beyond sowing streets (anhydrous ammonia or UAN flocks in the center of the streets), a little nitrogen should be readily available, so the crop will not become deficient in nitrogen before gaining access to the main source of its supply [53].

2.3.2. Nitrogen fertilizers and environmental problems

Fertilizers are chemical compounds used in conventional agriculture to increase the amount of nutrients in the soil and, consequently, achieve a productivity gain. Currently, they are very used, although not everyone agrees with their use [9].

Mainly, the problem of fertilizers lies in their impacts beyond the food chain. These include soil quality degradation, water and air sources pollution and increased pest resistance [4].

There are two major groups of fertilizers: inorganic and organic. Both can be natural or synthetic [5].

2.3.2.1. Inorganic fertilizers

The most common inorganic fertilizers carry nitrogen, phosphate, potassium, magnesium or sulfur and the major advantage of these types of fertilizers is that they contain large concentrations of nutrients that can be absorbed almost instantaneously by the plants [46].

Nitrogen fertilizers are among the most used and are the ones that cause the greatest environmental impact [70]. The production of these compounds is responsible for 94% of the energy consumption of the entire fertilizer production. The main fuels used are natural gas (73%) and coal (27%), both fossils, whose carbon dioxide (CO_2) emissions contribute to the imbalance processes of the greenhouse effect, thus favoring global warming. Manufacturing consumes approximately 5% of the annual production of natural gas [71].

In general, the use of inorganic fertilizers causes environmental problems, among them the contamination of groundwater, rivers and lakes. Many inorganic fertilizers carry persistent organic pollutants (POPs), such as dioxins and heavy metals, in their composition, which contaminates animals and plants that live in the water. Other animals or humans themselves may become contaminated by drinking water or eating intoxicated animals [72].

Water contamination may also lead to its eutrophication. This is a process in which, according to studies, when nitrogen or phosphate compounds reach rivers, lakes and coastal areas, they favor the growth and increase numbers of algae, what leads to an oxygen decrease and death of various organisms. Some environmentalists claim that this process creates "dead zones" in aquatic environments, with no life beyond seaweed [65].

Studies show that phosphate and nitrogen fertilizers can also cause soil dependence by killing organisms from their microflora, such as mycorrhiza fungus and various bacteria that contribute to soil richness and plant development. Acidification is also one of the problems and would cause soil nutrients loss [69].

2.3.2.2. Organic fertilizers

Organic fertilizers are made from natural products, such as humus, bone meal, castor oil cake, seaweed and manure [7]. Studies show that the use of organic fertilizers increases soil biodiversity, with the emergence of microorganisms and fungi that contribute to plant growth. In addition, in the long run, there is an increase in soil productivity, unlike what happens with conventional inorganic fertilizers [73].

Other researches claim that one of the dangers of organic fertilizers is in their own composition. If not manufactured correctly, they may contain pathogens [74].

The amount of nutrients present in organic fertilizers is not accurate and, unlike inorganic fertilizers, may not be readily available at the right time for plant growth. This means that there is no use for this type of fertilizer in modern intensive agricultural production [71].

Although on a much smaller scale, this type of fertilizer, as well as inorganic ones, cause soil acidification and can release nitrous oxide into the atmosphere [4].

Over time, organic fertilizers have become less widely used and there is no research that can provide good funding for replacing inorganic fertilizers with less abrasive chemicals in the environment [69].

2.4. SOIL CONDITIONERS

Soil conditioners are products that promote the improvement of the physical, physicochemical and/or biological properties of the soil [75].

In Brazil, these products may be composed of several sources released for use by the Ministry of Agriculture, Livestock and Food Supply (MAPA), however, the origin of each different classifications carries different uses.

According to the Normative Instructions 25/2009 [76] and 35/2006 [77], the Ministry of Agriculture, Livestock and Supply (MAPA), Section V. Art. 6 Soil conditioners will be classified, according to their raw materials, in six different classes:

- Class A, a product which uses raw material of plant, animal or agro-processing origin where no sodium (Na^+), heavy metals, elements or potentially toxic synthetic organic compounds are used in the process; Released for use in greenery, orchards, gardens and there is no need to use gloves and masks in its application. Product suitable for planting vegetables, orchards, ornamental plants, flowers and gardens;
- Class B, a product that uses raw material from industrial processing or agro-industry where sodium (Na^+), heavy metals, elements or potentially toxic synthetic organic compounds are used in the process; Prohibited products for use in greenhouses and orchards. Individual Protective Equipment (gloves, apron, glasses, hat and masks) must be used in its application;

- Class C, a product that uses any amount of raw material from its household waste, resulting in a safe use in agriculture; Prohibited products for use in greenhouses and orchards. Individual Protective Equipment (gloves, apron, glasses, hat and masks) must be used in its application. Products of this class may only be marketed to final consumers, upon technical recommendation signed by an agronomist or forestry engineer;
- Class D, a product that uses any quantity of raw material from the treatment of sanitary waste, resulting in a safe use in agriculture; Application only with mechanized equipment (application of limestone and plaster in depth). Personal Protective Equipment (PPE) should be used during handling and application. Use prohibited in grazing and cultivation of greenhouses (vegetables), tubers, roots and flooded crops (rice), as well as other crops that the edible part meets the soil. Products of this class may only be marketed to final consumers, upon technical recommendation signed by an agronomist or forestry engineer;
- Class E, product that exclusively uses raw material of mineral or chemical origin;
- Class F, product which in its manufacture uses in any proportion the mixture of raw materials of the products of Classes A and E.

The use of soil conditioners is a promising technique, despite the low utilization and research, highlighting the use of hydro-retentive polymers, capable of increasing the water storage capacity in the soil and contributing to the growth and development of the crop [78].

2.4.1. Biochar

The name biochar comes from the junction of two English words: biomass and charcoal. It is obtained from organic plant or animal matter. The factors that interfere in the final product are temperature, pyrolysis time and raw material used [79].

The idea of biochar arose from studies of the organic matter of the Black Indian Lands, Amazonian soils altered by human presence with excellent agronomic and environmental characteristics. In addition to the high fertility, they present high stable carbon content (of pyrogenic origin, that is, produced by fire or heat) in their organic fraction, which provided a soil model suitable for carbon sequestration. The knowledge of its structure and its properties has enabled the search for materials and techniques that aim to imitate it in agricultural practices [79].

The application of biochar in soils has several objectives, highlighting, in addition to increased agricultural profitability and increased carbon sequestration, reduced fertilizer use, pollution risk management and environmental eutrophication, and restoration of degraded areas [10].

The response to the use of biochar in agricultural crops varies according to the soil characteristics. In general, crops have been performing well, resulting in reduced greenhouse gas emissions and reduced eutrophication in aquatic environments [11].

2.4.2. Zeolites

Zeolites are minerals that, because of their physical and chemical characteristics, are very widely used. Its main uses in agriculture are [12]:

- Apatite solubilizing agent for phosphate fertilization;
- Agricultural soil conditioner;
- Formulation of herbicides, insecticides and fungicides;
- Production of organomineral fertilizers;
- Production of slow release fertilizers;
- Artificial substrate for plant cultivation.

The three main properties of zeolites are the high cation exchange capacity, the high capacity of free water retention in the channels and the high ability to capture ions. These properties give it great interest for agricultural use [78].

In agriculture, the addition of zeolites to the soil increases their cation exchange capacity, which results in an increase in nutrient retention and pH, as well as in the improvement of their physical characteristics. The zeolites' cation exchange capacity improves fertilizer efficiency and reduces nutrient leaching due to the fact that the essential elements for the plants are retained on the surface and released slowly to absorb them [10].

In environmental protection the presence of zeolites in the soil can also delay or reduce nutrients leaching in plant's roots, contributing to reduce their migration to the water and thus minimizing the eutrophication processes of the water sources. In addition, if the NH_4^+ ion is held at exchangeable sites of the zeolites so that it is not accessible to the micro-organisms can reduce the volatilization of the nitrogen gas [11].

2.4.3. Mineral fertilizers

Mineral fertilizers are removed from natural mines and undergo transformations in chemical laboratories, they may contain one or more chemical elements. The main elements used for mineral fertilization are potassium, nitrogen and phosphorus. When applied to plants, they are naturally absorbed or undergo some changes to be fully assimilated. They can be found in powder, granules or bran [80].

3. METHODOLOGY

3.1. SITE CARACHTERIZATION

The field work was installed in Polytechnic Institute of Bragança's experimental farm (Poulão farm), located in the rural area of Bragança's municipality, with coordinates 41°46'48.59"N and 6°47'54.83"W and altitude of 704 meters as shown in Figure 2.

Figure 2: Field experiment conducted in Poulão farm [81].

The pot experiment was carried out in the greenhouse area of the Polytechnic Institute of Bragança - IPB, with coordinates 41°47'48.46"N and 6°45'43.25"W and altitude of 674 meters, the main area can be seen in Figure 3.

Figure 3: Polytechnic Institute of Bragança's greenhouse area [81].

This region presents an average annual temperature of 12.8 °C. In August, the hottest month, the average temperature is 23.4 °C. In the coldest month, February, the average temperature is 4.5 °C. The average annual rainfall is 706.31 mm, with the wettest month (March) averaging 193.77 mm and the driest month (August) 0 mm. The mean monthly temperature and precipitation recorded during 2018 can be observed in Figure 4.

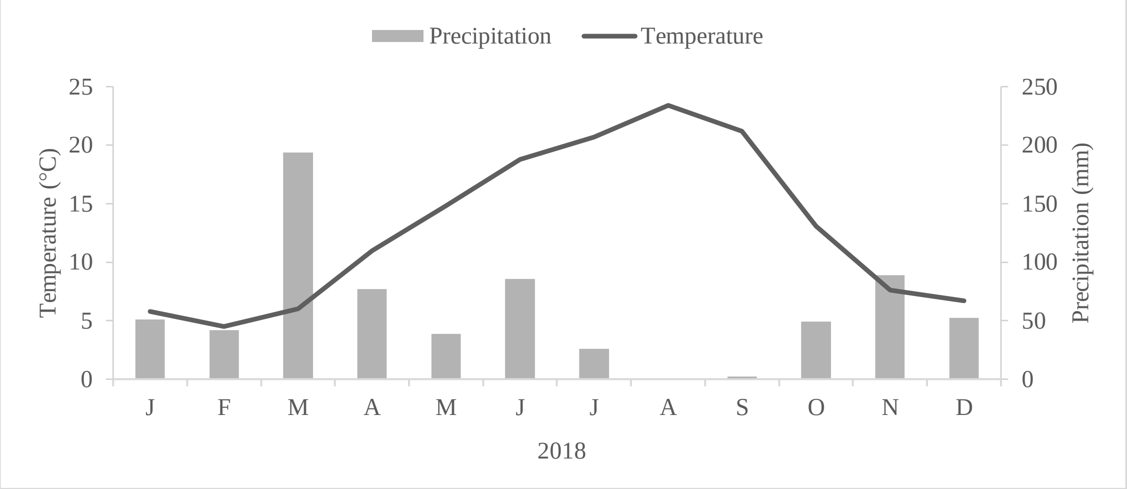


Figure 4: Average monthly temperature and precipitation in 2018, recorded in Santa Apolónia farm in Bragança.

According to the Koppen classification, Bragança benefits from a Csb (temperate climate with dry and mild summer) climate.

For the field experiment soil samples were taken from a depth of 0-20 cm, all over the main area, before the implementation of the experiment, for soil characterization. The soil was classified as eutric Fluvisol of sandy clay loam texture, containing 54% of sand, 25% of silt and 21% of clay.

The soil of the pot experiment is an eutric Regosol of colluvial origin, silt loam textured. Other physico-chemical properties of this soil were determined from soil samples collected before the implantation of the experiment at a depth of 0-20 cm. The soil used in the pot experiment was sieved in a 2 cm mesh and the subsamples carried out to the laboratory were dried at 40 ºC and sieved in a 2 mm mesh. Table 1 shows some chemical properties determined in these subsamples.

Table 1: Soil chemical properties from samples collected at a depth of 0-20 cm before the pot and field experiments implementation.

Properties	Pot Experiment	Field Experiment	Properties	Pot Experiment	Field Experiment
pH $_{H2O}$	6.54	5.54	Ca (cmol kg^{-1})[5]	9.08	10.93
pH $_{KCl}$	5.31	4.64	Mg (cmol kg^{-1})[5]	4.45	6.03
OM (g kg^{-1})[1]	1.20	2.17	K (cmol kg^{-1})[5]	0.25	0.2
N (g kg^{-1})[2]	-	1.17	Na (cmol kg-1)[5]	0.77	0.21
P_2O_5 (mg kg^{-1})[3]	16	26	EA (cmol kg^{-1})[5]	0.00	0.23
K_2O (mg kg^{-1})[3]	78	63	Al (cmol kg^{-1})[5]	0.00	0.03
Boron (mg kg^{-1})[4]	0.61	0.47	CEC (cmol kg^{-1})[5]	14.55	17.61

[1] Organic Matter, Walkley-Black; [2] Kjedalhl. [3] Egner-Rhiem; [4] Azometine-H; [5] Ammonium acetate pH 7.0,

The soil conditioners used were biochar, sold with the commercial name of Ecochar, derived from acacia (*Acacia mimosa*) pruning wood biomass, pyrolyzed at 600 °C. The zeolites used are a commercial product of the brand Fertcel. The main properties of these materials are presented in Table 2.

Table 2: Properties of the soil conditioners used in the experiments

Zeolites		**Biochar**	
SiO_2 (%)	63	**Total Organic Carbon (%)**	90
TiO_2 (%)	0.45	**Ashes (%)**	5
Al_2O_3 (%)	11.6	**Humidity (%)**	30
Fe_2O_3 (%)	1.81	**Nitrogen total (%)**	0.5
FeO (%)	0.81	**Volatile (%)**	5
MgO (%)	0.92	**Cadmium (mg kg^{-1})**	0.05
CaO (%)	5.78	**Lead (mg kg^{-1})**	0.05
Na_2O (%)	2.39	**Iron (mg kg^{-1})**	99.5
K_2O (%)	1.49	**Arsenic (mg kg^{-1})**	0.1
P_2O_5 (%)	0.09	**Mercury (mg kg^{-1})**	0.1
H_2O (%)	3.44	**Conductivity (µS/cm)**	948
Specific weight (g/cm3)	2.1	**TMC[2] (col./g)**	1.0*10^1
BD[1] (g/cm3)	0.98	**BD[1] (kg/m3)**	350
pH	7.6	**pH**	9

[1] Bulk Density, [2] Total Micologic Content.

The mineral fertilizers used was the ammonium nitrate (27% N), superphosphate (18% P_2O_5) and potassium chloride (60% K_2O). Nitrogen is the element used in the experimental design (at variable rates), whereas phosphorus and potassium were used in the same rates in all plots as a basal fertilization plan.

3.2. EXPERIMENTAL DESIGNS

3.2.1. Field Experiment

The field experiment was outlined with 36 plots of 4x5m size. The treatments were arranged in a full factorial design. The factor soil conditioners included biochar, zeolites and mineral (absence of conditioner). The factor nitrogen fertilization included the rates of 0, 50 (25 + 25), 100 (50 + 50) and 200 (100 + 100) kg N ha^{-1}, split into equal rates at preplant and sidedress. From each combination of factors three replicates were included. Biochar was used at a rate corresponding to the application of 10 t ha^{-1}. For zeolites the corresponding rate was 5 t ha^{-1}. All plots received P (P_2O_5) and K (K_2O) at 150 kg ha^{-1} at preplant as a basal fertilization plan.

The soil conditioners and fertilizers were weighed in the rates corresponding to each experimental unit and homogeneously distributed in the respective plot according to the experimental design. Thereafter the materials were incorporated into the soil and the corn (hybrid ‘Monero’, FAO 500) was sown in the next day, spaced at 0.7 x 0.18 m.

After fifty-one days there was a soil collection for determination of pre-sidedress soil nitrate test and to apply the nitrogen rate that corresponds to the sidedress application. At sidedress nitrogen application, the crop displayed the aspect shown in Figure 5.

Figure 5: Corn plants in a vegetative stage, around V3 and V4 (20 to 30 cm), when sidedress nitrogen was applied.

The nutritional status of the plants was monitored by SPAD and NDVI readings on July 28th at the V7 phenological stage. In the same date, leaf samples were collected to monitor the nutritional status of plants by means of leaf analysis in the laboratory.

At the one hundredth day, samples of 0.7 square meters, around six plants, from each plot were harvested and weighted fresh. A subsample representing an entire plant was carried to the lab, weighed fresh, oven dried at 65 ºC, weighed again and ground. A subsample of the basal portion of the stalks was also separated from the plants carried to the lab, dried and ground. The subsamples of the whole plant were used for elemental analysis and stalks for nitrate concentration.

3.2.2. Pot Experiment

The pot experiment consisted in twenty-four pots, with six treatments, in a completely randomized experimental design and four replicates of each treatment. The treatments were:

- Soil (Control)
- Soil + Biochar
- Soil + Zeolites
- Soil + Biochar + N
- Soil + Zeolites + N
- Soil + N

The pots had approximately 8 kg of soil and for the Biochar, Zeolites and Nitrogen fertilization, each pot received, respectively, 10 t ha^{-1}, 5 t ha^{-1} and 0.2 t ha^{-1}.

In each pot, three seeds were planted. As they developed, the ones that got smaller and less healthy were thinned to a single plant per pot. The pot experiment can be observed in Figure 6.

Figure 6: Pots containing one plant

In the beginning of October, the plants from all the pots were harvested and taken to the laboratory. They were oven dried at 65 ºC, weighed, ground and analyzed for elemental composition.

3.3. CROP HUSBANDRY

3.3.1. Field Experiment

The field experiment started with a soil preparation done by a moldboard plow and followed by a cultivator. In each plot a different soil conditioner or fertilizer were applied according to the experimental design. Fertilizers and soil conditioners were incorporated with a last pass of the cultivator.

The area counted on a central pivot to help on its irrigation, therefore it had a weekly irrigation of 40 mm when the rain was not enough to supply the crop demand, so the crop was watered during June, July and August. There was a total of approximately 400 mm water during the whole growing season.

There was also a onetime weed control when the plants were in their vegetative state (4 to 6 leaves). The product applied was a post-emergence foliar absorption herbicide (Laudis), which formulation is an oil dispersion of 22 g/L isoxadifene-ethyl and 44 g/L tembotrione. It was applied at the concentration of 0.5 L (hL and 2L ha^{-1}).

3.3.2. Pot Experiment

To conduct the pot experiment there was a soil collection of around 8 kg of soil per pot on the area next to the greenhouse. The collected soil was then put on each pot, after had been sieved in a 2 cm mesh, along with the fertilizers and soil conditioners. The conditioners and fertilizers applied in each treatment and pot are described in Table 3.

Table 3: Conditioners and fertilizers applied in each pot.

Treatment	Biochar (g pot^{-1})	Zeolites (g pot^{-1})	Nitrogen (g pot^{-1})
Control	0	0	0
Biochar	4.46	0	0
Zeolites	0	2.23	0
Biochar + N	4.46	0	2
Zeolites + N	0	2.23	2
N	0	0	2

In the early stages of the crop, the pots were watered 3 times per week, but as the summer got closer and the plants got bigger, watering became a daily task. The pots received around 500 ml every day.

The weed control was made manually every time they started to grow and compete with the corn plants. The area around the pots was moaned as soon as the corn plants started to show their first leaves, so the weeds around the area would not get in the way of the corn's growth.

During the experiment plant's height was measured twice, in order to better monitor their growth and development when exposed to different treatments.

3.4. FIELD SAMPLING AND IN SITU MEASUREMENTS

During the field experiment, a soil sample was collected, when the plants were around V3 and V4 stages in order to perform the Pre-sidedress soil nitrate test (PSNT). This test must be done when the plants are between 15 and 30 cm, because in this period there is a greater nitrogen requirement to support its rapid growth spurt.

SPAD, NDVI and Fluorometer measurements were taken both in field and pots experiments. On the field, the measurements were taken on July 28th, along with leaves collection. For the pots' trials, these indexes were measured twice. The first measurement was taken on July 31st and the second one happened on August 14th.

3.4.1. SPAD

The SPAD (Soil Plant Analysis Development) -502 is a portable equipment that estimates the chlorophyll content by measuring the transmittance of the light through the leaf.

Fast and easy measurement of chlorophyll levels in plant leaves without damaging the leaf. Measurements are made only by closing the measuring head on the leaf. Since the leaf is not cut or otherwise damaged, the same leaf can be measured as the plant grows.

3.4.2. NDVI

The Normalized Difference Vegetation Index (NDVI) is an index that analyzes vegetation condition through remote sensing, comparing the amount of infrared and red light that is reflected by the leaves of the plants. The equipment used to collect this data was the FIELDSCOUT® CM1000 meter.

NDVI index measures on a range of -1 to 1, so as the index collected comes closer to 1, the healthier the plants are, as observed in Figure 7.

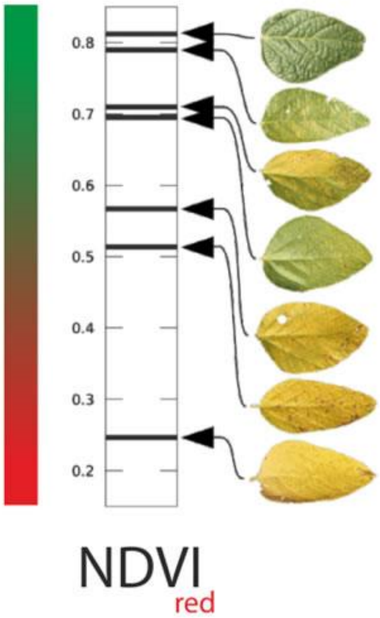


Figure 7: NDVI ratio in plants leaves [82].

3.4.3. Fluorometer

The Fluorometer is a portable continuous excitation system for analyzing chlorophyll *a* fluorescence, transient fluorescence and the OJIP transients, it is a great tool for evaluating plant stress and the way it affects photosystem II.

F_M, F_0 and F_V are, respectively, maximum, minimum and variable fluorescence from dark adapted leaves. The F_v/F_m and F_v/F_0 transients show the maximum potential quantum efficiency of photosystem II, but F_v/F_0 is a much more sensitive measurement than F_v/F_m due to the fact that it is normalized over the minimum fluorescence instead of the maximum. The OJIP transients provides the origin fluorescence (O) at 20 µs, fluorescence, at 2 ms (J) and 30 ms (I) and the maximum fluorescence (P or F_m).

The used equipment was an OS30P+ Fluorometer. It works as a small equipment that is put on the leaf of the plant previously submitted to darkness and then observe the results shown on screen and download the content to better analyze all the data collected.

3.5. LABORATORY ANALYSIS

All the procedures done for the laboratorial analysis occurred in Polytechnic Institute of Bragança’s Plant and Soil laboratory located in the school of agriculture and on Mountain Research Center (CIMO).

3.5.1. Plant analysis

Plant analysis were conducted in both, field and pot experiment, except for the stalk nitrate test, that was measured only in stalks from the end of the corn crop on the field experiment. Plant collection occurred twice in the field experiment, the first one when the crop was at V7 stage and the second one in the end of the crop cycle.

3.5.1.1. Plant preparation

Before proceeding to the laboratorial analysis, all the leaves, stalks and whole plants were weighted and put on the oven at around 65 ºC. After they were dried out, they were weighted again to calculate their total dry biomass and then taken to a mill. After being milled the samples were taken to the laboratory to start their analysis.

3.5.1.2. Nitrogen concentration in plant tissues

To determine the nitrogen concentration, the Kjeldahl procedure was used, which is divided into two steps: the first one consists in the digestion of the sample for the conversion of the organic nitrogen in NH_4^+ -N.

For this analysis, one gram of the samples was weighed and put into digestion tubes, then 15 ml of concentrated sulfuric acid and two pellets of a selenium catalyst were added. The samples were then placed in a digestion block at a temperature of 400 °C for 40 minutes. Heating the sample with sulfuric acid promotes oxidation and the presence of the catalyst increases the rate of oxidation of the organic matter by the acid.

The second step consists in the determination of the NH_4^+ -N. After the digestion and the cooling period, the tubes were sent to the Kjeltec TM 8400 Autoanalyser FOSS equipment where the nitrogen concentration was given by the distillation of the digested part with sodium hydroxide, forming ammonia and entraining in a stream of vapor, determining the values of the titration with hydrochloric acid in a solution containing boric acid.

3.5.1.3. Boron concentration in plant tissue

For the boron concentration, 1 g of the milled plant was weighted and put on a small container along with 0.10 g of calcium oxide (CaO). After that, the samples were taken to a heating plate at 200 ºC until they were toasted. The toasted samples were taken to an oven at 500 ºC for one and a half hour. After the samples were taken off the oven and were getting colder, sulfuric and boric acid were added, along with distilled water and then filtered in order to get the final sample for the analysis.

To proceed with the analysis, five standard solutions were prepared, growing linearly from 1 to 5 µg of boron, including a sixth solution containing $CaCl_2$ for the 0 µg of B/ml. All samples were then taken to a spectrophotometer reading at a 420 nm wavelength and correctly analyzed in this range.

3.5.1.4. Phosphorus, potassium, calcium, magnesium, copper, iron, zinc and manganese concentration in plant tissue

To determine tissue phosphorus, potassium, calcium, magnesium, copper, iron, zinc and manganese concentration, 0.25 g of dry matter was weighed into digestion tubes suitable for the microwave digestion equipment (CEM MARS XPRESS), with 10 ml of concentrated nitric acid. After the nitric digestion the solution was diluted in 50 ml of deionized water to determine the values of these components.

The calcium, magnesium, copper, iron, zinc and manganese cations were determined by atomic absorption spectrophotometry and potassium by flame emission spectrophotometry in a UNICAM equipment. Phosphorus was determined after promoting the development of a blue color using the blue molybdate method and ascorbic acid as a reducing agent. In this determination a GENESYS spectrophotometer and wavelength of 882 nm were used.

3.5.1.5. Stalk nitrate test

Before starting the Stalk Nitrate Test, all 36 stalk samples were dried and milled. 2 g of these milled samples were weighted and put along with 50 ml of distilled water in an agitator for 30 minutes. These samples were filtered, and their extracts were put in the spectrophotometer and analyzed in two wavelengths: 220 nm to read NO_3^- and 275 nm to determine dissolved organic matter interference.

3.5.2. Soil analysis

Soil analyses were conducted only in the field experiment. The samples were taken twice, the first one on mid-cycle, when plants were at V3 to V4 stage to perform the pre-sidedress soil nitrate test (PSNT) and the second time in the end of the crop cycle. All soil methodologies followed the Soil test interpretation guide [83].

3.5.2.1. Soil preparation

To conduct soil analysis, the collected soil was previously frozen, and, after a while, every sample was separated in two different plastic bags. The first bags were weighted and put on a hothouse. This soil was then sifted at a 2-milimeter mesh and ready to be analyzed. The second bags stood on the refrigerator and, after some time were sifted at a 2-milimeter mesh while they were still frozen. These bags were taken back to the refrigerator, where they stood until they were analyzed.

3.5.2.2. Phosphorus and potassium determination

To determine soil phosphorus and potassium extractable forms, there was an extraction by a combination of ammonium lactate and acetic acid, at a pH of 3.7 in a soil fraction, in the ratio of 1:5. This solution was then stirred at 180 revolutions per minute (rpm) for 2 hours. After this period the samples were filtered, and the phosphorus and potassium were determined in the extract.

The phosphate was determined calorimetrically with the blue ammonium molybdate method along with the reducing agent ascorbic acid. Potassium was quantified by flame photometry on a JENWAY flame photometer.

3.5.2.3. pH

The pH of the soils was determined in a soil and water suspension and in a soil and KCl 1M solution. The soil/solution ratio was 1:2.5. The soil remained in contact with the solutions for 2 hours. At the end of this time, a potentiometer was used with a glass electrode to determine the pH of the samples.

3.5.2.4. Boron extraction

Boron extraction in soils was taken by the hot water method, determined by calorimetry. First, 10 g of soil were weighted and put on plastic bags. These bags were then sealed with a $CaCl_2$ 0.01M solution inside, in order to mitigate the errors due to the yellow coloration. These solutions were out in contact with the hot water and, after 20 minutes, they were filtered into small pots to get the main extract of the samples. Subsequently the extracts were mixed with azomethine-H complexing agent of $B(OH)_3$ in aqueous medium.

3.5.2.5. Cation exchange capacity

The cation exchange capacity is the sum of the named cation exchange bases (Ca^{2+}, Mg_2^+, K^+ and Na^+) and exchange acidity (Al_2^+ and H^+). This soil property was determined after weighing 2.5 g of soil and adding 50 ml of the buffered ammonium acetate solution at pH 7.0 and shaken for 30 minutes. At the end of this time the solution was filtered and in the filtered extract the values for the Ca^{2+} and Mg^{2+} concentration was found by atomic absorption spectrometry and the ones for K^+ and Na^+ were determined by flame emission spectrometry in a PYE UNICAM, model PU9100X.

3.5.2.6. Exchange acidity

To determine the exchange acidity, 10 g of soil were put with 100 ml of a KCl 1M solution in the agitator for 30 minutes. After this time the sample was filtered and titrated with 0.1 M NaOH and phenolphthalein as indicator. The results found in this titration are the exchange acidity values.

3.5.2.7. Organic matter

The organic matter was determined by the Walkley-Black analytical method, which consists in a wet digestion of the soil organic matter with potassium dichromate and sulfuric acid for 30 minutes. This procedure was made to evaluate the easily oxidizable organic carbon. After this period the residual dichromate is titrated with the ammoniacal iron sulphate. The carbon present in the sample is quantified by the amount of unreacted potassium dichromate. The organic matter content is estimated by multiplying the carbon percentage by the factor of 1.72, based on the estimate that the organic matter of the soil contains 58% of carbon.

3.5.2.8. Pre-sidedress soil nitrate test

In order to start the PSNT, 10 g of frozen soil were weighted and put on plastic bottles along with 40 ml of a KCl solution. These bottles were taken to an agitator where they stood for 1 hour. After that, these soil plus KCl solutions were filtered into small pots, where they received 1 ml of solution for each 14 ml of KCl.

Then, standard solutions were made for a standard curve of NO_3^- in a range of 0-7 mg NO_3-N/L (0 to 350 µg of NO_3-N/50 mL.

These solutions were then taken to a spectrophotometer and analyzed in two wavelengths: 220 nm to read NO_3^- and 275 nm to determine dissolved organic matter interference.

3.5.2.9. Ammonium concentration

For the ammonium concentration two procedures were done. The first one by using a cold KCl solution. It consisted in 10 g of frozen soil that were weighted and put with 40 ml of the cold KCl solution into plastic bottles. These bottles were agitated for one hour and filtered into small pots.

The second procedure consisted in the same first steps as the cold KCl, but after the soil plus 40 ml of the KCl was taken off the agitator, the solution was taken into a hothouse where it stood for 4 hours at 100 °C. The solutions were filtered as well into small pots.

The ammonium concentration is a blue solution formed by the reaction of ammonia, hypochlorite and phenol, catalyzed by sodium nitroprusside. For that to be done, 10 ml of the soil plus KCl (both cold and hot) solution were mixed with 0.4 ml of phenol, 0.4 ml of the sodium nitroprusside solution and 1 ml of the oxidant solution. After adding all the reagents, the solution stood covered for an hour, until the blue color could be fully developed.

Standard solutions were prepared in a range of 0-0.6 mg N/L, diluting the N-NH_4 solution volume.

These solutions were then taken to a spectrophotometer and analyzed in the 640 nm wavelengths, to determine its absorbance and then calculate the ammonium concentration of the soil samples.

3.6. DATA ANALYSIS

The data was submitted to variance analysis in the JPM trial 14 program. When significant differences occurred, the means were separated by the Tukey (HSD) multiple comparison test ($\alpha = 0.05$).

Apparent nitrogen recovery (ANR) was estimated as an index of nitrogen use efficiency by using the following equation:

$$ANR = \frac{NEf - NEt}{NAf}\, x\, 100$$

ANR – Apparent nitrogen recovery

NEf - Nutrient recovery in the fertilized plants

NEt - Nutrient recovery in the control plants

NAf – Nutrient applied as a fertilizer

4. RESULTS

4.1. INORGANIC NITROGEN IN SOIL AT PRE-SIDEDRESS

4.1.1. Soil mineral nitrogen at pre-sidedress

Pre-sidedress soil nitrate test was conducted in mid-cycle corn. There was found significant interaction between soil conditioners and nitrogen rates, as well as significant differences among soil conditioners and among nitrogen rates (Figure 8).

Mineral fertilizers presented the higher soil nitrate concentrations, with an average value of 91.6 mg kg^{-1}, and Biochar the lower concentration, with 34.0 mg kg^{-1}. Among the different nitrogen rates, 200 kg N ha^{-1} showed higher soil nitrate concentration, with 67 mg kg^{-1} as average value and 50 kg N ha^{-1} the lower value, with 44 mg kg^{-1}.

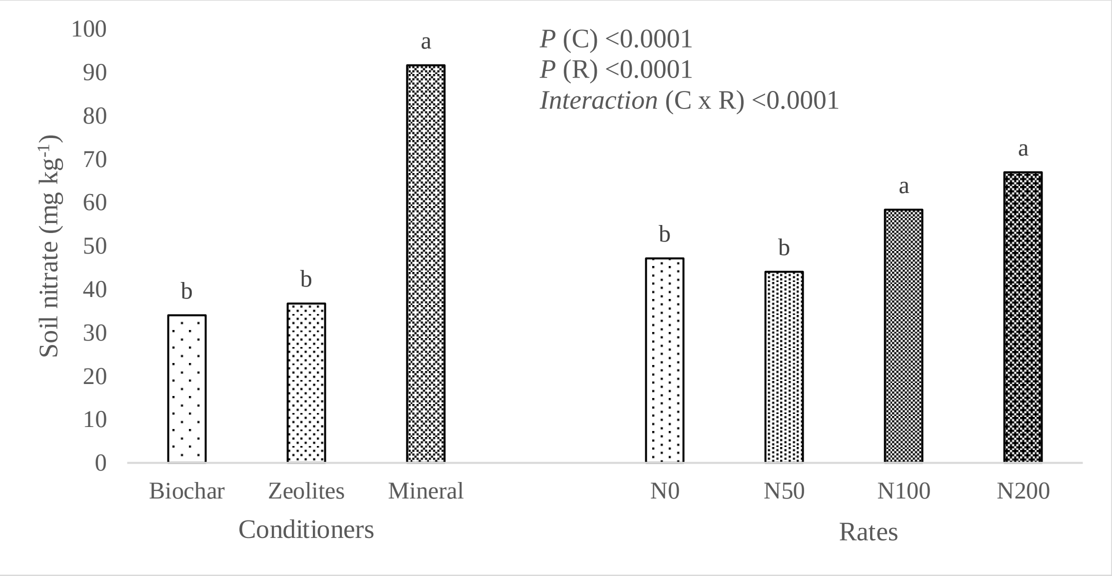


Figure 8: Pre-sidedress soil nitrate test in mid-cycle of corn in the field experiment, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

There was found significant interaction between soil conditioners and nitrogen rates in soil ammonium concentration determined in mid-cycle of corn (Figure 9). They were also found significant differences among soil conditioners but not among nitrogen rates in soil ammonium levels.

Among the soil conditioners, Mineral fertilizers had the higher ammonium concentration, with 2.56 mg kg^{-1}, and Biochar the lower concentration, with 2.05 mg kg^{-1}.

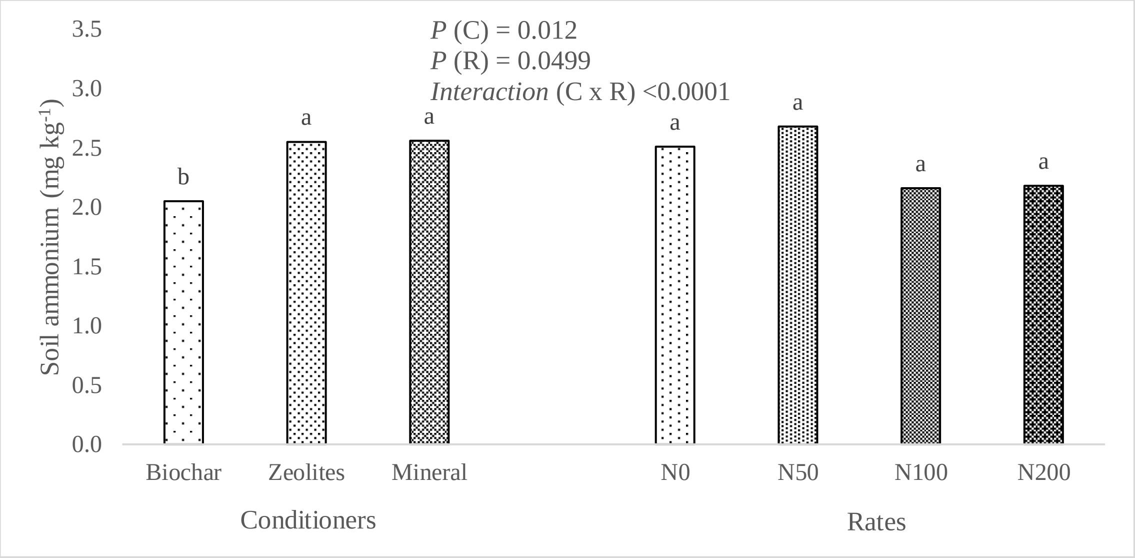


Figure 9: Ammonium concentration in the soil in mid-cycle of corn in the field experiment, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2. PLANT DEVELOPMENT AND NUTRITIONAL STATUS INDICATORS DURING THE GROWING SEASON

4.2.1. SPAD

The measurements from the SPAD – 502 equipment occurred both in the field experiment and in the pot experiment. In the field experiment there was significant interaction between the two factors, soil conditioners and nitrogen rates and significant differences when these two factors were analyzed separately (Figure 10).

Among soil conditioners, Biochar was the treatment that showed lower average value (56.6) and Mineral fertilizer was the treatment showing the higher average value (59.3). The SPAD values increased significantly as the nitrogen rates increased (N0, N50, N100 and N200). The nitrogen rate that showed greater values was 200 kg N ha^{-1} with an average value of 61.9.

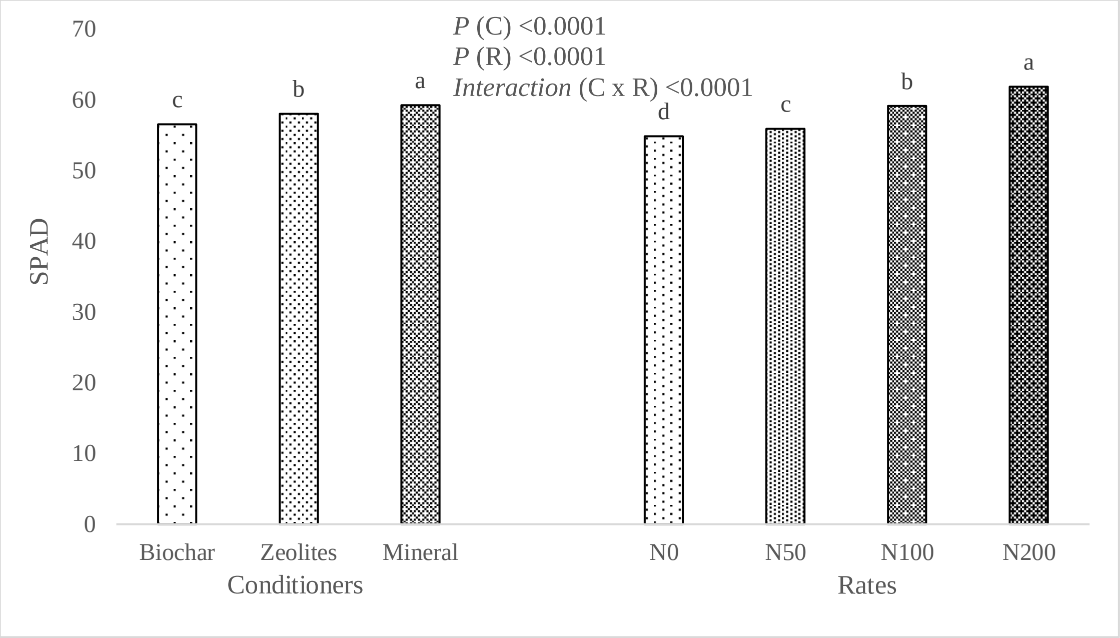


Figure 10: SPAD measurement taken on July 28th in the field experiment, as a function of soil conditioners (C) and nitrogen rates (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

SPAD-readings were taken in two dates in the pot experiment. The first measurement, on July 31st (Figure 11) showed significant differences among the treatments that received nitrogen in comparison to those that did not receive nitrogen.

The treatments that received nitrogen presented higher values, with the highest found on the Zeolites + N treatment (39.4) and the lower value found on the Biochar + N treatment (38.4).

The treatments that did not receive nitrogen presented lower results among all the treatments. The plants of the Control treatment showed the lower results with an average of 26.1 and those of the Zeolites had the highest average value with 28.3.

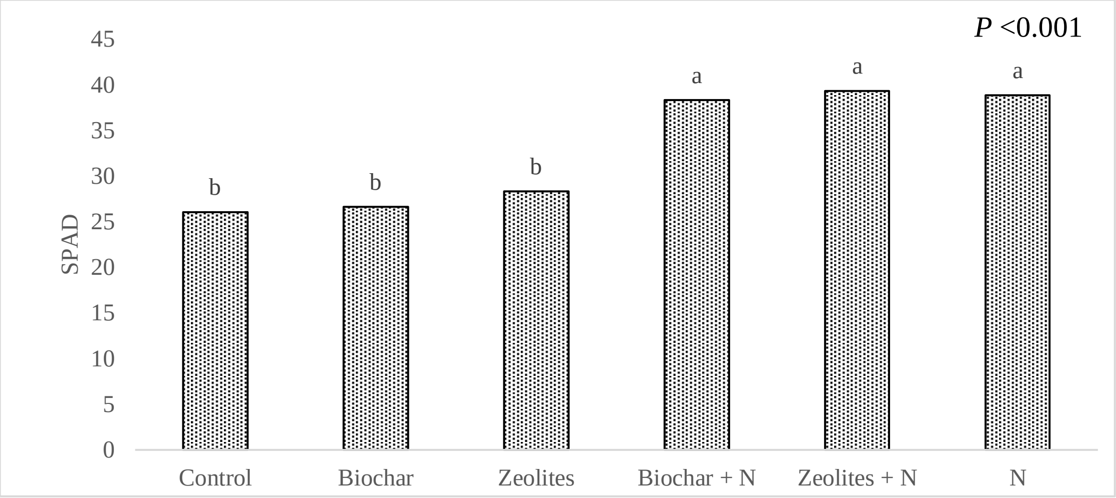


Figure 11: SPAD measurement taken on July 31st in the pot experiment The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

The second SPAD measurements occurred on August 14th. There were found significant differences between the treatments that received nitrogen and the ones that did not (Figure 12). The treatments that received nitrogen showed the higher values. The results varied from 39.5 (N alone) to 42.7 (Zeolites + N).

The values of the treatments that were not supplied with nitrogen were significantly lower. Biochar used alone had the lower average value, with 13.2, followed by the Control treatment, with 14.4, and the Zeolites used alone, with 15.4.

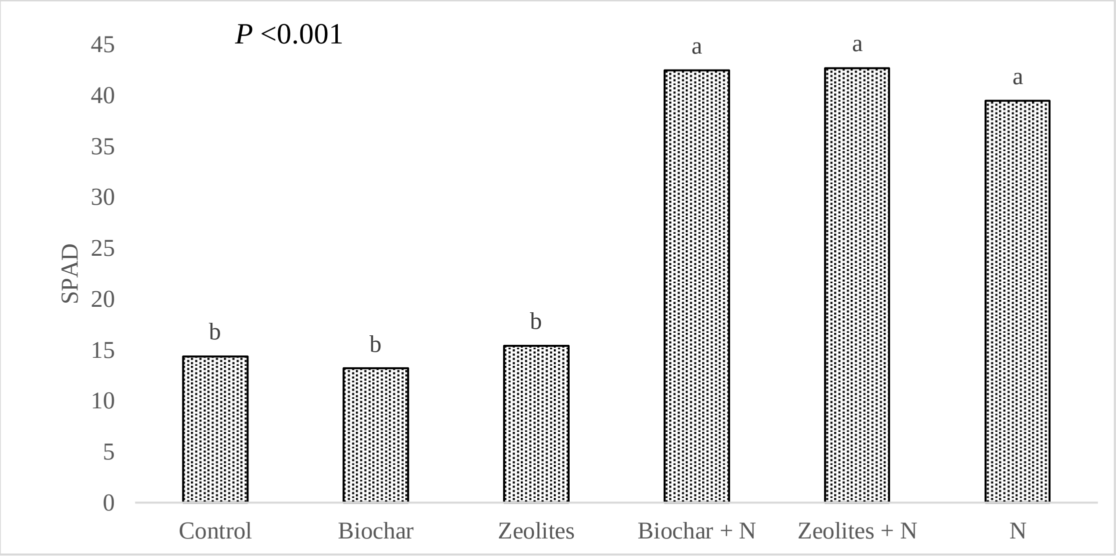


Figure 12: SPAD measurement taken on August 14th in the pot experiment. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2.2. NDVI

For the field experiment, the values of NDVI are presented in Figure 13. There was not found significant interaction between soil conditioners and nitrogen rates, nor between soil conditioners or between nitrogen rates. Even though the conditioners showed a low *P* value (0.0593) the results could not be considered significantly different between treatments at $P < 0.05$. Among soil conditioners, the Zeolites presented the lower average value (0.811) and the Biochar had the higher average value (0.833).

There was not found a consistent pattern of NDVI as the nitrogen rate increased. The nitrogen rate showed the higher average value was 100 kg N ha^{-1} with 0.827 and the rate with lower results was 200 kg N ha^{-1} with an average of 0.820.

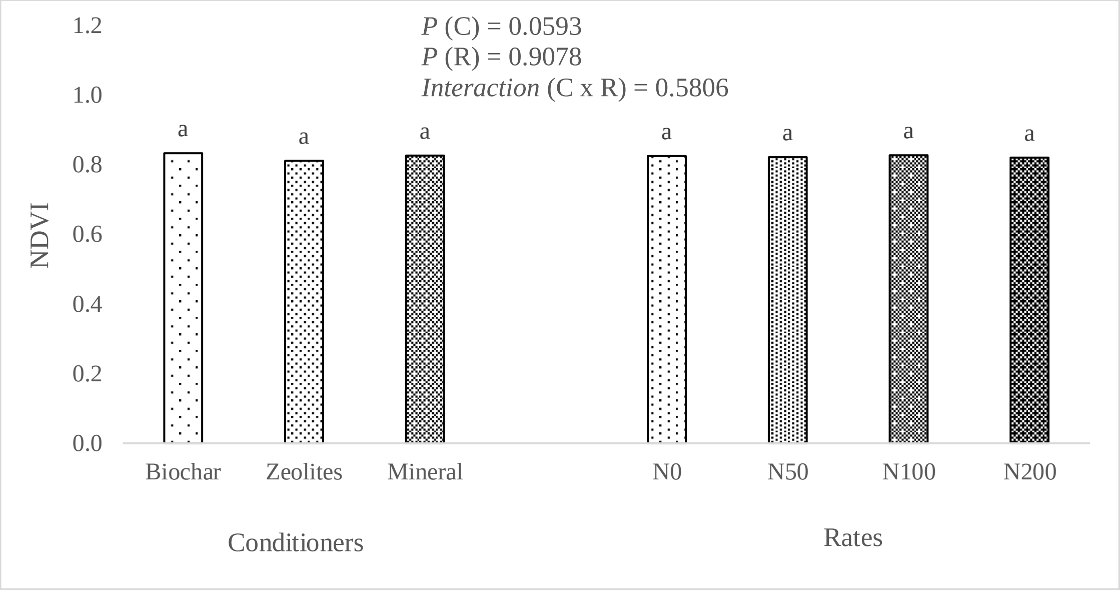


Figure 13: Normalized difference vegetation index (NDVI) measured on July 28th in the field experiment, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

In pot experiment, the first measurement of NDVI occurred on July 31st and no significant differences between the treatments were found (Figure 14).

Biochar used alone presented the lower average NDVI value (0.620), but when nitrogen was added (Biochar + N), NDVI reached the highest average value among the treatments (0.730).

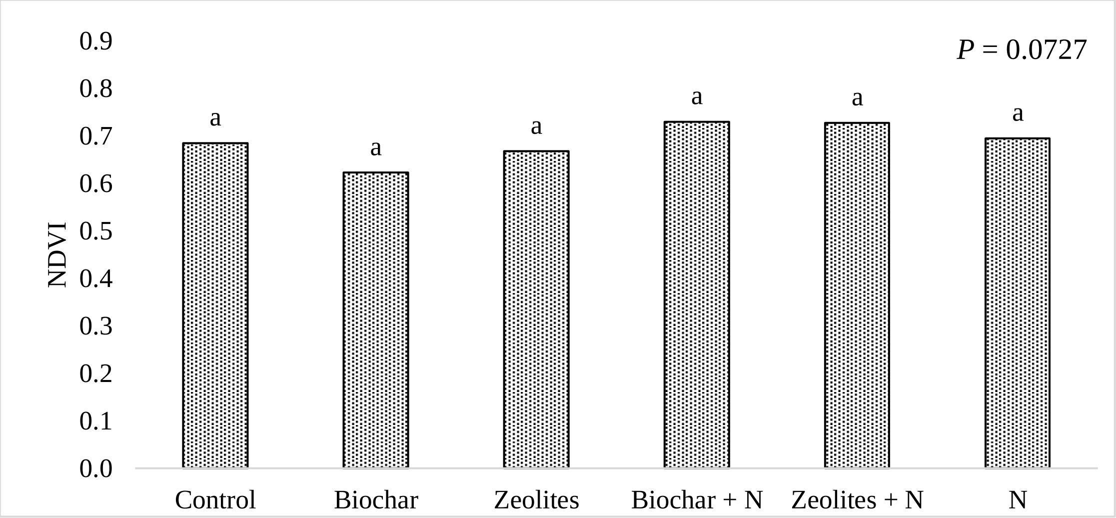


Figure 14: Normalized difference vegetation index (NDVI) measured on July 31st. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

The second measurements of NDVI in the pot experiment were taken on August 14th. In this second date of records, the results showed significant differences between the group of treatments receiving nitrogen and the group that did not receive (Figure 15).

Zeolites used alone gave the lower average value (0.620). However, when the nitrogen was added, the Zeolites + N treatment presented the higher results, with an index of 0.748.

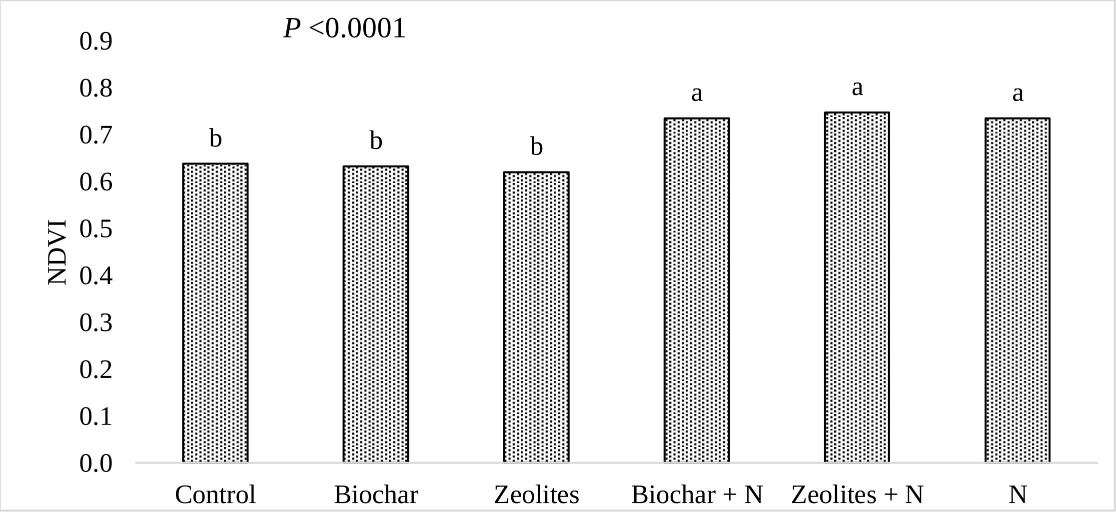


Figure 15: Normalized difference vegetation index (NDVI) measured on August 14th in the pot experiment. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2.3. Fluorometer

The measurements taken in the field with the fluorometer OS30P+ did not reveal significant interaction among soil conditioners and nitrogen rates, nor significant differences between soil conditioners or nitrogen rates for any of the variable analyzed (Table 4).

Biochar presented higher average values for the OJIP transients, while the use of mineral fertilization without soil conditioner had higher average values of F_v/F_m and F_v/F_0.

There was observed a slight increase on the OJIP transient from 0 kg N ha^{-1} to 100 kg N ha^{-1}, but these values decreased in the treatment 200 kg N ha^{-1}. Average values of F_v/F_m and F_v/F_0 were higher when 100 kg N ha^{-1} were applied, with the respective values of 0.762 and 3269.3.

Table 4: Fluorometer measurement in the field experiment.

	O	**J**	**I**	**P**	**F_v/F_m**	**F_v/F_0**
Biochar	277.4 a[*]	369.5 a	519.0 a	721.4 a	0.754 a	3136.6 a
Zeolites	255.8 a	336.9 a	449.3 a	671.0 a	0.745 a	2979.4 a
Mineral	267.5 a	364.6 a	505.5 a	719.1 a	0.758 a	3183.6 a
N0	268.3 a	350.8 a	482.7 a	704.3 a	0.749 a	3037.0 a
N50	269.8 a	361.0 a	500.2 a	710.0 a	0.747 a	2994.7 a
N100	277.2 a	375.5 a	530.3 a	725.0 a	0.762 a	3269.3 a
N200	252.2 a	340.7 a	451.8 a	676.0 a	0.753 a	3098.5 a
***P* (Conditioners)**	0.2990	0.1138	0.1152	0.1743	0.6717	0.7103
***P* (Rate)**	0.4472	0.2902	0.2556	0.5226	0.8284	0.7994
***Interaction* (CxR)**	0.2233	0.0238	0.0245	0.0294	0.4218	0.3904

[*] The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

In the pot experiment the chlorophyll fluorescence was measured two times, the first one on July 31st (Table 5) and the second on August 14th (Table 6). On the first measurement, the OJIP, F_v/F_m and F_v/F_0 transients did not show significant differences among treatments.

Between the treatments that received nitrogen, Zeolites + N had higher average results in the OJIP transient variables, however the F_v/F_m and F_v/F_0 values were higher on the nitrogen used alone treatment.

The treatments that did not receive the extra nitrogen dosage had lower average results when compared to the treatments that received nitrogen, nevertheless, among these treatments, there were similar results on the OJIP transient. The F_v/F_m values were similar for treatments where the Biochar and Zeolites were used alone (0.755) while for the F_v/F_0, the Zeolites alone presented the higher average value, with 3104.0.

Table 5: Fluorometer measurement in the pot experiment on July 31st.

	O	**J**	**I**	**P**	**F_v/F_m**	**F_v/F_0**
Control	253.0 a*	340.0 a	474.0 a	581.5 a	0.749 a	3017.0 a
Biochar	246.5 a	331.5 a	457.0 a	582.0 a	0.755 a	3098.5 a
Zeolites	243.5 a	336.0 a	476.0 a	593.0 a	0.755 a	3104.0 a
Biochar + N	243.5 a	339.0 a	468.5 a	669.5 a	0.760 a	3184.0 a
Zeolites + N	277.0 a	365.0 a	514.0 a	697.0 a	0.750 a	3013.0 a
N	266.0 a	352.0 a	514.0 a	702.0 a	0.763 a	3233.5 a
P	0.6670	0.1732	0.3030	0.0225	0.8772	0.8892

*The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

The second date of measurement revealed significant differences among treatments for all the variables analyzed, except F_v/F_m (Table 6) The Zeolites + N treatment presented higher results in all the variables, and the pots containing Biochar used alone showed lower results in almost all variables, except from the origin fluorescence (O) at 20 µs, when the Zeolites used alone presented the lower value (206.5).

Table 6: Fluorometer measurement in the pot experiment on August 14th.

	O	J	I	P	F_v/F_m	F_v/F_0
Control	213.0 bc*	278.5 b	364.0 b	437.5 b	0.713 a	2501.0 bc
Biochar	212.5 bc	271.5 b	356.5 b	429.5 b	0.701 a	2418.5 c
Zeolites	206.5 c	283.0 b	375.0 b	444.0 b	0.712 a	2490.5 bc
Biochar + N	266.0 abc	364.0 a	542.0 a	765.0 a	0.761 a	3193.5 abc
Zeolites + N	275.5 a	396.0 a	585.0 a	786.5 a	0.773 a	3405.5 a
N	267.0 ab	373.5 a	557.5 a	768.0 a	0.771 a	3375.5 ab
P	0.0105	0.0004	0.0008	0.0002	0.0283	0.0097

*The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2.4. Nitrogen concentration in plant leaves at V7 stage

Nitrogen concentration was measured in the leaves at the V7 stage, in the field experiment, as presented in Figure 16.

It was found significant interaction between soil conditioners and nitrogen treatments, which means that the response of conditioners depends on the rate of nitrogen applied and vice versa.

There were also found significant differences among the soil conditioners. Zeolites presented the higher average value with 30.1 g kg^{-1} and Mineral Fertilizers the lower value with 26.3 g kg^{-1}. For the different nitrogen rates, the two most fertilized treatments showed the higher values. The highest average value was found with the application of 100 kg N ha^{-1} (30.3 g kg^{-1}) and the lowest in the control treatment (25.6 g kg^{-1}).

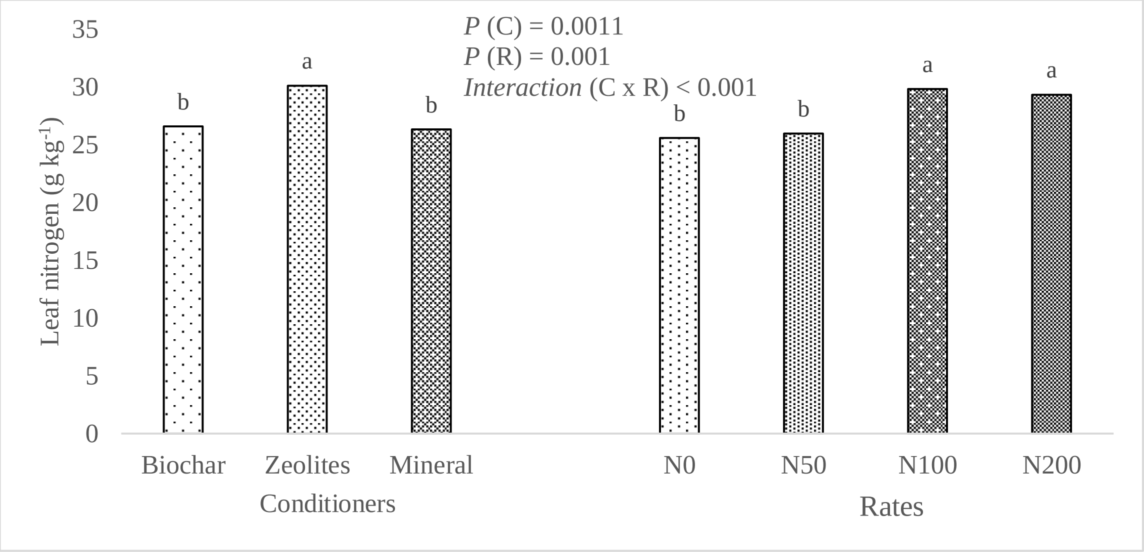


Figure 16: Nitrogen concentration on corn leaves at the V7 stage, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2.5. Phosphorus, potassium, calcium and magnesium in plant leaves at V7 stage

Phosphorus, potassium, calcium and magnesium measurement occurred on the leaves in the V7 stage (Table 7).

Leaf phosphorus concentration did not show significant interaction between soil conditioners and nitrogen rates or significant differences among the different nitrogen rates (0, 50, 100 and 200 kg N ha^{-1}), but when comparing the values obtained from the soil conditioners, they presented significant differences. Zeolites was the treatment with higher average value (2.6 g kg^{-1}) and Biochar was the treatment with lower leaf phosphorus concentrations, with an average value of 1.9 g kg^{-1}.

For leaf potassium concentrations, the conditioners presented significant differences among them. Mineral fertilizers showed the higher results, with 17.6 g kg^{-1}, followed by Biochar, with 16.7 g kg^{-1} and, with the lower results, the Zeolites, with 15.9 g kg^{-1}. The different nitrogen rates showed significant differences among them, the treatment with 0 kg N ha^{-1} presented the higher potassium percentage, with 17.4 g kg^{-1} and the treatment with 100 kg N ha^{-1} the lower percentage, with 14.8 g kg^{-1}. There was observed significant interaction between soil conditioners and nitrogen rates for leaf potassium concentration.

For leaf calcium concentration there were found significant differences among soil conditioners and an interaction between them and the nitrogen rates. Mineral fertilizers had the higher values, with 5.5 g kg^{-1} and Zeolites the lower, with 3.2 g kg^{-1}. There were not recorded significant differences among nitrogen rates.

Magnesium concentrations presented significant differences between both, soil conditioners and nitrogen rates (Table 7). It was also observed significant interaction among the two factors. For the soil conditioners, Mineral fertilizers showed higher values, with 5.9 g kg^{-1}, followed by the Zeolites, with 5.6 g kg^{-1} and Biochar, with 5.1 g kg^{-1}. Among the different nitrogen rates, the one with higher average leaf magnesium concentration was 0 kg N ha$^{-1,}$ with 6.0 g kg^{-1}, followed by 100 kg N ha^{-1}, with 5.8 g kg^{-1}, 50 kg N ha^{-1}, with 5.5 g kg^{-1} and. The lower results were found with 200 kg N ha^{-1} (4.8 g kg^{-1}).

Table 7: Leaf phosphorus, potassium, calcium and magnesium concentrations in corn leaves at V7 stage.

	P	**K**	**Ca**	**Mg**
	----------------------------	**g kg^{-1}**	----------------------------	
Biochar	1.94 b*	16.75 ab	3.58 b	5.11 b
Zeolites	2.56 a	15.93 b	3.19 c	5.58 ab
Mineral	2.04 b	17.60 a	5.54 a	5.86 a
N0	2.07 a	17.38 a	4.20 a	5.95 a
N50	2.19 a	17.50 a	4.07 a	5.47 ab
N100	2.19 a	14.82 b	3.95 a	5.81 a
N200	2.27 a	17.32 a	4.12 a	4.84 b
***P* (Conditioners)**	<0.0001	0.0337	<0.0001	0.0272
***P* (Rate)**	0.3700	0.0016	0.3821	0.0061
***Interaction* (C x R)**	0.2900	<0.0001	0.0293	0.0178

*In columns, separated by soil conditioner and nitrogen rates, the same letter indicates the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.2.6. Micronutrients in plant leaves at V7 stage

In the field experiment the micronutrients were determined twice, the first time on corn leaves when the crop was at the V7 stage and the second time at harvest in the whole plant. In the pot experiment the concentration of micronutrients in plant tissues were determined only at harvest.

Iron, zinc and manganese are the micronutrients in higher concentration on the leaves, respectively (Table 8). Leaf iron concentration presented significant interaction between soil conditioners and nitrogen rates and significant differences among soil conditioners and nitrogen rates. Zeolites was the soil conditioner with higher leaf iron concentration, reaching 160.4 mg kg^{-1}, Mineral fertilizers presented lower iron concentration in the leaves, with 101.5 mg kg^{-1}. Among the different nitrogen rates the higher average leaf iron concentration was found on 200 kg N ha^{-1} (149.9 mg kg^{-1}), and the lower concentration on 50 kg N ha^{-1} (107.9 mg kg^{-1}).

Leaf zinc concentration presented significant interaction between conditioners and nitrogen rates, as well as significant differences among the soil conditioners, but not among nitrogen rates. For the different soil conditioners, Zeolites is the one that outstands among them, with 68.5 mg kg^{-1}. On the other hand, Mineral fertilizers presented lower results, with 45.3 mg kg^{-1}.

For leaf manganese concentration there was found significant interaction between soil conditioners and nitrogen rates and significant differences among soil conditioners and nitrogen rates. Among the soil conditioners, Zeolites was the one with higher average leaf manganese concentration (54.4 mg kg^{-1}), and Biochar was the one with lower average value (42.4 mg kg^{-1}). For the different nitrogen rates, 0 kg N ha^{-1} gave the higher manganese concentration, with 51.9 mg kg^{-1}, and 100 kg N ha^{-1} gave the lower concentration, with 44.1 mg kg^{-1}.

Boron concentration presented an interaction between conditioners and nitrogen rates, and these factors were significantly different among them. Among the conditioners, the higher average values were found in Mineral fertilizers (13.1 mg kg^{-1}), and the lower in Biochar (11.2 mg kg^{-1}). As for the different nitrogen rates, 200 kg N ha^{-1} showed higher average values, with 13.0 mg kg^{-1}, and 100 kg N ha^{-1} the lower average concentration, with 10.5 mg kg^{-1}.

Table 8: Concentration of boron (B), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in corn leaves at V7 stage in the field experiment as a function of soil conditioner (C) and nitrogen rate (R).

	B	**Fe**	**Mn**	**Zn**	**Cu**
	----------------------------**mg kg^{-1}**----------------------------				
Biochar	11.2 b*	128.9 b	42.4 b	61.9 a	15.0 c
Zeolites	12.3 ab	160.4 a	54.4 a	68.5 a	16.4 b
Mineral	13.1 a	101.5 c	51.1 a	45.3 b	17.9 a
N0	12.9 a	114.9 b	51.9 a	55.1 a	15.5 b
N50	12.3 a	107.9 b	51.3 a	60.9 a	16.2 ab
N100	10.5 b	148.4 a	44.1 b	61.3 a	17.3 a
N200	13.0 a	149.9 a	49.8 a	57.1 a	16.7 ab
***P* (Conditioner)**	0.0011	<0.0001	<0.0001	<0.0001	<0.0001
***P* (Rate)**	0.0002	<0.0001	0.0002	0.2206	0.0412
***Interaction* (C x R)**	<0.0001	<0.0001	0.0001	0.0107	0.01

*In columns, separated by soil conditioner and nitrogen rates, the same letter indicates the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.2.7. Plant height

Plant height was only measured in the pot experiment. There were no significant differences among the treatments on the first measurement (July 31st). The higher average value, in this measurement, was found in the Zeolites + N treatment (0.83 m) and the lower average value was found in the Biochar used alone treatment (0.76 m). The results found on July 31st can be seen on Figure 17.

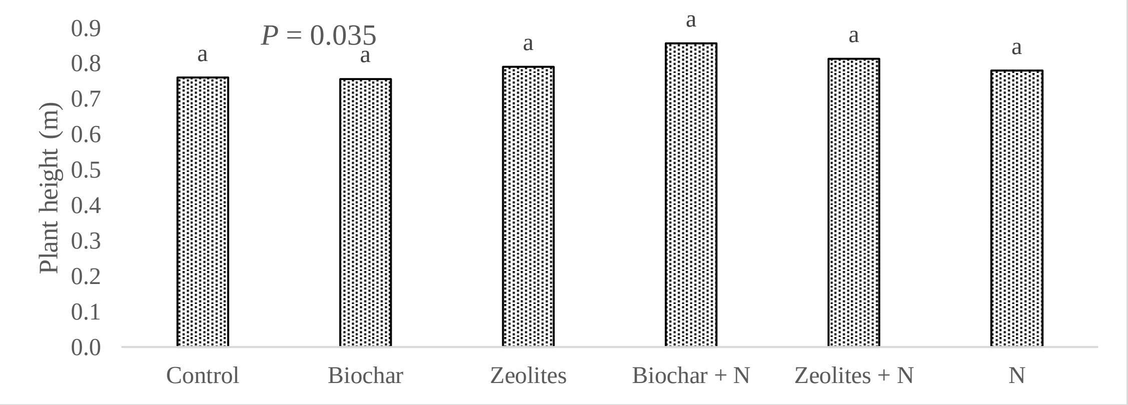


Figure 17: Corn height in the pot experiment on July 31st. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

The second measurement of plant height (August 14th) presented significant differences among the treatments. The treatments that did not receive the extra nitrogen dosage had lower values (0.84 to 0.90 m) and the treatments that received the extra nitrogen dosage had significantly higher values (1.06 to 1.19 m). The measured values on August 14th can be seen on Figure 18.

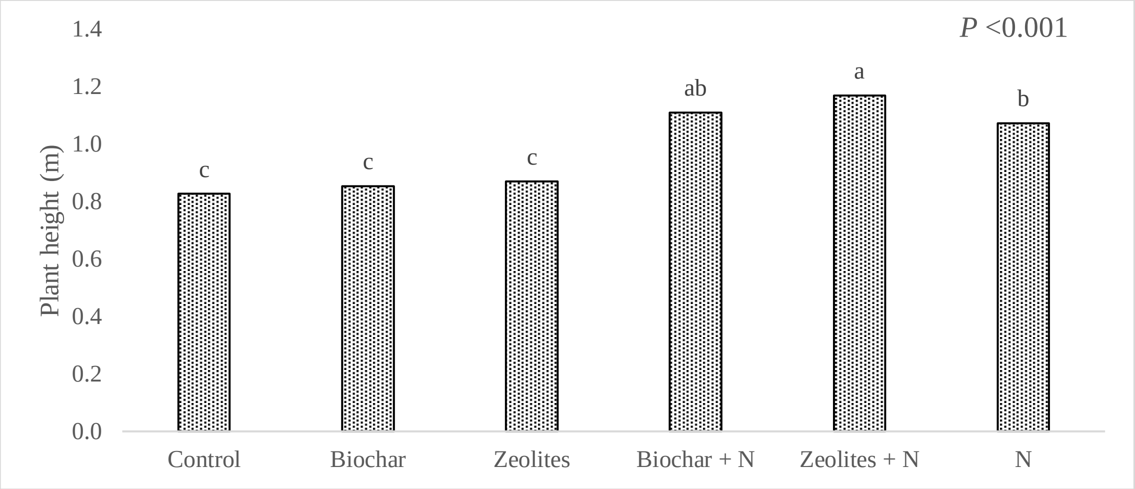


Figure 18: Corn height in the pot experiment on August 14th. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.3. DRY MATTER YIELD AND NUTRIENTS IN PLANT TISSUES AT HARVEST

4.3.1. Dry matter yield

Dry matter yield was measured both in the field and in the pot experiments. In the field experiment (Figure 19), no significant interaction was found between soil conditioners and nitrogen rates. However, there were found significant differences among the soil conditioners. The higher average value was found for Zeolites, with 15.7 t ha^{-1} and the lower value was found for Biochar, with 12.0 t ha^{-1}. As the nitrogen rate increased, dry matter yield grew simultaneously. Dry matter yield varied from 11.7 t ha^{-1} to 17.0 t ha^{-1} between N0 and N200.

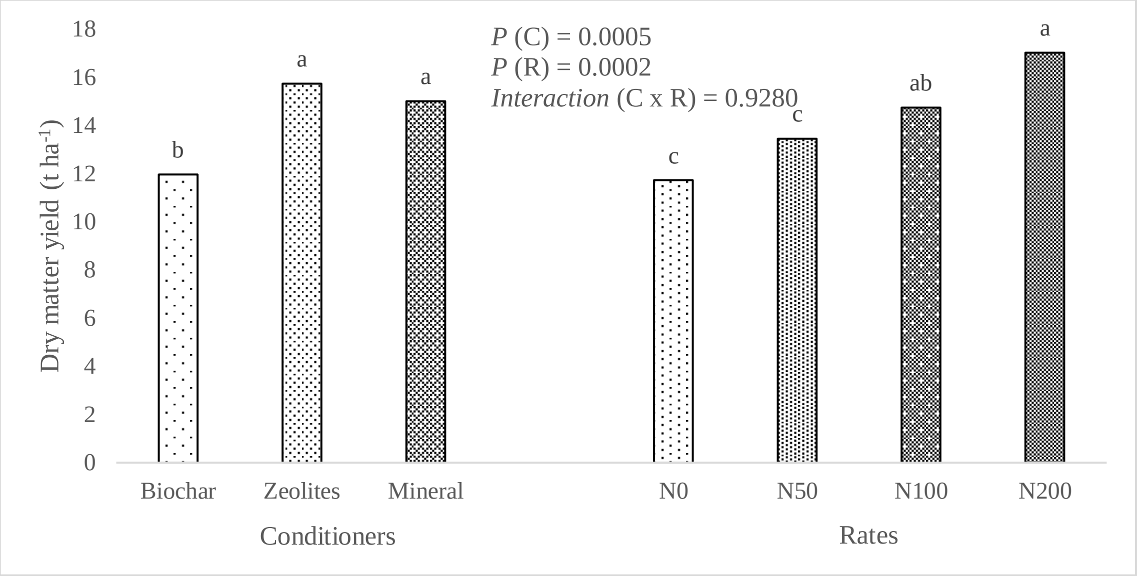


Figure 19: Dry matter yield in the field experiment as a function of soil conditioners (C) and nitrogen rates (R) [0 (N0), 50 (N50), 100 (N100) and 200 (N200) kg ha^{-1}]. For each experimental factor (soil conditioners or nitrogen rates) the same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

In the pot experiment (Figure 20), the pots that received an extra dosage of nitrogen presented higher dry matter yields. The highest average value was found in the Biochar + N treatment (51.1 g plant^{-1}). In all the experiment the lowest average value was found with Biochar used alone (28.4 g plant^{-1}).

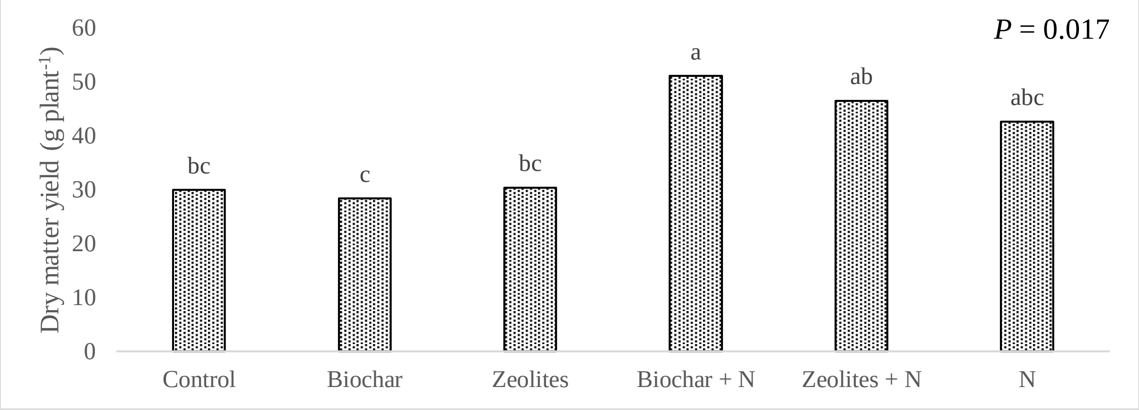


Figure 20: Dry matter yield in the pot experiment. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.3.2. Nitrogen concentration in plant tissues

Nitrogen concentration in plant tissues was determined both in the field and in the pot experiments.

The nitrogen concentration was determined at the harvest in the aboveground biomass (Figure 21). It was also found significant interaction between soil conditioners and nitrogen rates. Among soil conditioners, there were not found significant differences, but the Zeolites treatment presented the higher average value, with 12.6 g kg^{-1}, and the Mineral Fertilizers treatment the lower value, with 11.8 g kg^{-1}. Among the different nitrogen rates, there were found significant differences, with the lower value (10.7 g kg^{-1}) found in the control treatment.

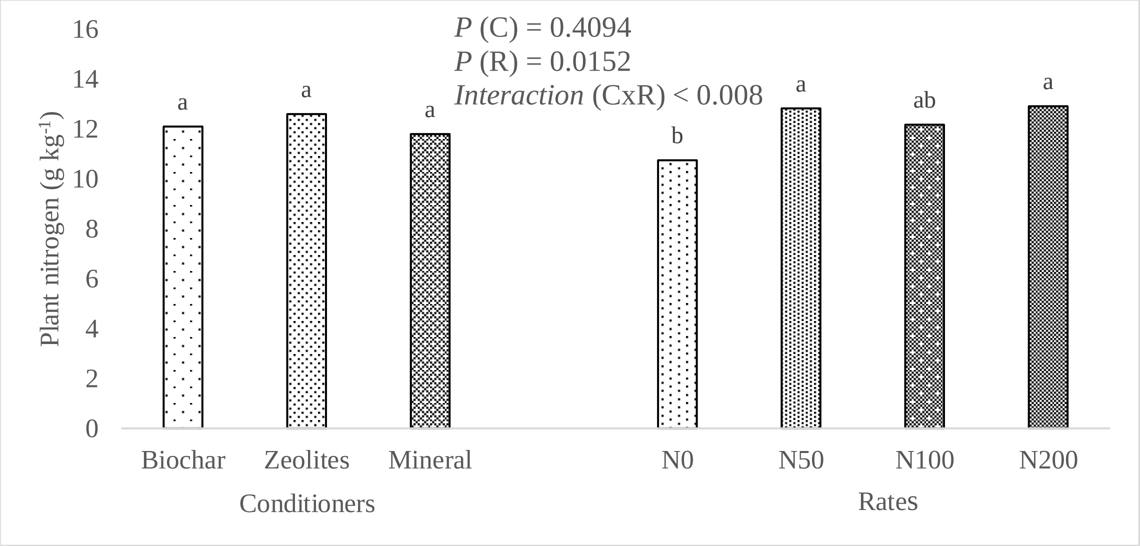


Figure 21: Nitrogen concentration in aboveground dry biomass at harvest, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

In the pot experiment the corn plants (aboveground dry matter) were analyzed for nitrogen concentration only at harvest. There were found significant differences between the treatments that received nitrogen and those that did not receive (Figure 22). Thus, Control, Biochar used alone, and Zeolites used alone treatments presented the lower values (respectively 8.4, 8.6 and 8.9 g kg^{-1}). The treatments that received the extra nitrogen dosage had the higher values. Nitrogen used alone produced the highest average nitrogen concentration (20.3 g kg^{-1}).

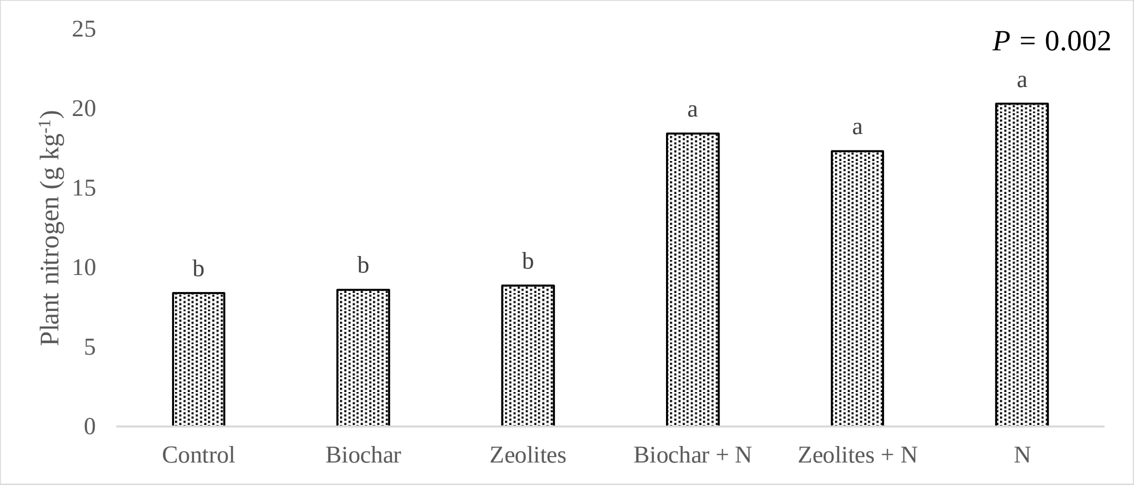


Figure 22: Nitrogen concentration in aboveground dry biomass in the pot experiment. The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.3.3. Phosphorus, potassium calcium and magnesium in plant tissues

The concentrations of phosphorus, potassium, calcium and magnesium in plant tissues were taken from both field and pot experiments.

For phosphorus concentration in the aboveground plant tissues there was not found significant interaction between soil conditioners and nitrogen rates (Table 9). Phosphorus concentration in the whole plant also did not show significant differences among the nitrogen rates. However, the soil conditioners presented significant differences among them. Biochar was the conditioner with higher phosphorus concentration in these tissues, with 1.3 g kg^{-1} and Mineral fertilizers the lower, with 1.1 g kg^{-1}.

Potassium concentrations in plant tissues presented significant interaction between soil conditioners and nitrogen rates and significant differences between both soil conditioners and nitrogen rates. For the conditioners, Biochar presented higher average value, with 13.1 g kg^{-1} and Mineral fertilizers the lower, with 10.0 g kg^{-1}. Among nitrogen rates the higher potassium concentrations were found with 50 kg N ha^{-1} (14.0 g kg^{-1}), and lower with 100 kg N ha^{-1} (9.9 g kg^{-1}).

For the concentration of calcium in plant tissues there was not found significant interaction between the factors under study nor significant differences among the nitrogen rates, but the soil conditioners presented significant differences among them. Zeolites was the conditioner with higher average value (3.4 g kg^{-1}), followed by Mineral fertilizers (3.2 g kg^{-1}) and the lower value was found in Biochar plots (2.6 g kg^{-1}).

Magnesium concentrations in plant tissues showed significant interaction between soil conditioners and nitrogen rates and significant differences among soil conditioners and among nitrogen rates. Mineral fertilizers presented the higher average value, with 4.1 g kg^{-1} and Zeolites the lower, with 3.4 g kg^{-1}. For the different nitrogen rates, the one that outstands was 100 kg N ha^{-1} (4.3 g kg^{-1}), followed by 0 kg N ha^{-1} (4.1 g kg^{-1}), 50 kg N ha^{-1} (3.4 g kg^{-1}) and, with the lower average value, 200 kg N ha^{-1} (3.1 g kg^{-1}).

Table 9: Phosphorus, potassium, calcium and magnesium concentrations in aboveground dry matter of corn plants in the field experiment.

	P	**K**	**Ca**	**Mg**
	---------------------------- **g kg^{-1}**			----------------------------
Biochar	1.28 a*	13.13 a	2.64 b	3.63 ab
Zeolites	1.25 a	12.01 ab	3.42 a	3.44 b
Mineral	1.10 b	10.00 b	3.25 a	4.11 a
N0	1.28 a	10.48 b	3.25 a	4.13 a
N50	1.25 a	13.96 a	2.82 a	3.42 b
N100	1.15 a	9.90 b	3.03 a	4.27 a
N200	1.14 a	12.52 ab	3.32 a	3.08 b
***P* (Conditioners)**	0.0083	0.0179	0.0001	0.0086
***P* (Rate)**	0.0928	0.0077	0.0527	<0.0001
***Interaction* (C x R)**	0.4311	0.4943	0.0003	0.0024

*In columns, separated by soil conditioner and nitrogen rates, the same letter indicates the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

In the pot experiment there were not found significant differences in phosphorus concentration in plant tissues among the treatments (Table 10). However, the Zeolites used alone treatment presented the higher average value, with 2.0 g kg^{-1}, followed by the Control treatment, with 1.9 g kg^{-1}. The lower value was found in the Nitrogen used alone treatment, with 1.1 g kg^{-1}.

The concentration of potassium in plant tissues also did not significantly vary among the treatments, nonetheless, Biochar + N showed the higher average value, with 30.0 g kg^{-1}, and the Control treatment the lower average value, with 22.7 g kg^{-1}.

Tissue calcium concentrations showed significant differences among the treatments. The treatments that did not receive an extra nitrogen dosage presented higher values, Zeolites used alone being the highest (3.0 g kg^{-1}), followed by Biochar used alone (2.6 g kg^{-1}) and Control treatment (2.0 g kg^{-1}). Between the treatments that received an extra nitrogen dosage, Nitrogen used alone presented the higher average value, with 1.6 g kg^{-1}, Zeolites + N had 1.4 g kg^{-1} and Biochar + N 1.3 g kg^{-1}.

The magnesium concentration in plant tissues significantly varied among the treatments, and the ones that received an extra nitrogen dosage presented higher values. Biochar + N treatment had the higher average value of 3.6 g kg^{-1}. Between the treatments that did not receive an extra nitrogen dosage, Zeolites had the higher average value, with 2.2 g kg^{-1}.

Table 10: Phosphorus, potassium, calcium and magnesium concentrations in the aboveground dry biomass in corn plants grown in the pot experiment.

	P	**K**	**Ca**	**Mg**
	---------------------------- **g kg^{-1}** ----------------------------			
Control	1.92 a*	22.36 a	1.96 ab	1.80 c
Biochar	1.66 a	24.71 a	2.61 ab	2.20 bc
Zeolites	1.97 a	23.72 a	3.04 a	2.21 bc
Biochar + N	1.55 a	30.06 a	1.38 b	3.61 a
Zeolites + N	1.45 a	27.68 a	1.42 b	3.15 ab
N	1.15 a	28.02 a	1.65 ab	3.28 ab
P	0.0720	0.1404	0.0188	0.0014

*The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.3.4. Micronutrients in plant tissues

The concentration of micronutrients in plant tissues was determined from both the field and pot experiments.

In the whole corn plants at the end of growing season, iron, zinc and manganese were the micronutrients in higher concentration, respectively (Table 11).

For the iron concentration in plant tissue, there was found significant interaction between conditioners and nitrogen rates, likewise significant differences within these two factors. Biochar was the treatment with higher average value (101.6 mg kg^{-1}), and Mineral fertilizers the one with the lower value (81.3 mg kg^{-1}). As for the different nitrogen treatments, 0 kg N ha^{-1} was the rate that presented higher tissue iron concentration, with an average of 129.3 mg kg^{-1}, and 200 kg N ha^{-1} was the rate with lower concentration, with 72.2 mg kg^{-1}.

There was not significant interaction for tissue zinc concentration between soil conditioners and nitrogen rates, but there were significant differences among soil conditioners and nitrogen rates. Zeolites was the treatment with higher average zinc concentration (37.8 mg kg^{-1}) and Mineral fertilizers the one with the lower average value (26.0 mg kg^{-1}). As for the different nitrogen rates, 50 kg N ha^{-1} was the one with higher values (37.0 mg kg^{-1}) and 200 kg N ha^{-1} with lower results (29.1 mg kg^{-1}).

As for tissue manganese concentration, there was observed significant interaction between factors, and significant differences among soil conditioners, but the nitrogen rates did not produce significant difference in tissue manganese concentrations. Mineral fertilizers outstand on manganese concentration, when compared to the other conditioners, its average value reach 31.2 mg kg^{-1}, while Biochar presents the lower value, with 24.5 mg kg^{-1}.

Boron concentration in plant tissues in the field experiment did not demonstrate any significant differences among conditioners and nitrogen rates, however there was an interaction between these factors. The higher boron concentration, among the conditioners, was found in Biochar, with 10.8 mg kg^{-1}, and the lower in Zeolites, with 9.4 mg kg^{-1}. Among the different nitrogen rates, 200 kg N ha^{-1} presented the higher average concentration (10.6 mg kg^{-1}), and 0 kg N ha^{-1} the lower (9.1 mg kg^{-1}).

Table 11: Concentrations of boron (B), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in corn plants in the field experiment as a function of soil conditioner (C) and nitrogen rate (R).

	B	**Fe**	**Mn**	**Zn**	**Cu**
			-----mg kg^{-1}-----		
Biochar	10.8 a	110.6 a	24.6 b	37.3 a	7.8 b
Zeolites	9.4 a	93.9 b	25.1 b	37.8 a	7.9 b
Mineral	9.8 a	81.3 c	31.2 a	26.0 b	11.3 a
N0	9.1 a	129.3 a	28.9 a	34.9 a	8.9 a
N50	9.9 a	101.9 b	26.0 a	37.0 a	9.3 a
N100	10.4 a	77.5 c	26.5 a	33.8 ab	9.3 a
N200	10.6 a	72.2 c	26.2 a	29.1 b	8.5 a
P **(C)**	0.0615	<0.0001	0.0004	<0.0001	<0.0001
P **(R)**	0.1037	<0.0001	0.343	0.003	0.2475
Interaction **(C x R)**	0.0004	<0.0001	0.0094	0.1127	0.0364

[*] In columns, separated by soil conditioner and nitrogen rates, the same letter indicates the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

In the pot experiment iron, manganese and zinc were, respectively, the micronutrients in higher concentrations in plant tissues. For all the micronutrients, the concentrations in plant tissues showed significant differences among the treatments (Table 12).

Iron concentration in plant tissue was higher in the Biochar used alone treatment, with 427.9 mg kg^{-1}, and the lower concentration was found in the Control treatment, with 210.8 mg kg^{-1}.

Tissue manganese concentration was higher on the treatments that received an extra nitrogen dosage. The higher value was found in Nitrogen used alone treatment, with 158.9 mg kg^{-1}, and the lower in the Biochar used alone treatment, with 49.9 mg kg^{-1}.

For the zinc concentration in plant tissue, the treatments that received an extra nitrogen dosage presented the higher values, Nitrogen used alone being the highest (158.8 mg kg^{-1}), followed by Zeolites + N (122.2 mg kg^{-1}) and Biochar + N (116.1 mg kg^{-1}). Among the treatments that did not receive and extra nitrogen dosage, Zeolites used alone was the treatment with higher values (34.9 mg kg^{-1}), followed by Biochar used alone (30.8 mg kg^{-1}) and Control treatment (28.5 mg kg^{-1}).

Boron average concentration was significantly different among the treatments. Nitrogen used alone demonstrated the higher boron average concentration, with 20.5 mg kg^{-1}, on the other side, Biochar + N had the lower average concentration, with 13.7 mg kg^{-1}.

Table 12: Concentrations of boron (B), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in corn plants in the pot experiment as a function of soil conditioner (C) and nitrogen rate (R).

	B	**Fe**	**Mn**	**Zn**	**Cu**
			----------------------------mg kg^{-1}----------------------------		
Control	17.7 ab	210.8 b	57.8 c	28.5 b	7.4 b
Biochar	16.9 ab	427.9 a	49.9 c	30.8 b	8.1 b
Zeolites	19.7 a	369.3 a	62.7 c	34.9 b	8.0 b
Biochar + N	13.7 b	407.3 a	116.1 b	81.8 a	12.0 a
Zeolites + N	17.4 ab	355.9 ab	122.2 b	77.4 a	10.5 a
N	20.5 a	412.4 a	158.9 a	88.9 a	10.9 a
P	0.0139	0.0044	<0.0001	<0.0001	<0.0001

*The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.4. SOIL PROPERTIES AT HARVEST

4.4.1. Soil organic matter at the end of the growing cycle

No significant interaction was found between soil conditioners and nitrogen rates in soil organic matter content (Figure 23). However, there were found significant differences among soil conditioners.

The plots treated with Biochar presented significantly higher levels of organic matter than the other treatments, reaching an average value of 25 g kg^{-1}. Zeolites gave significantly higher values than Mineral fertilizers. The average values were respectively 23 g kg^{-1} and 22 g kg^{-1}.

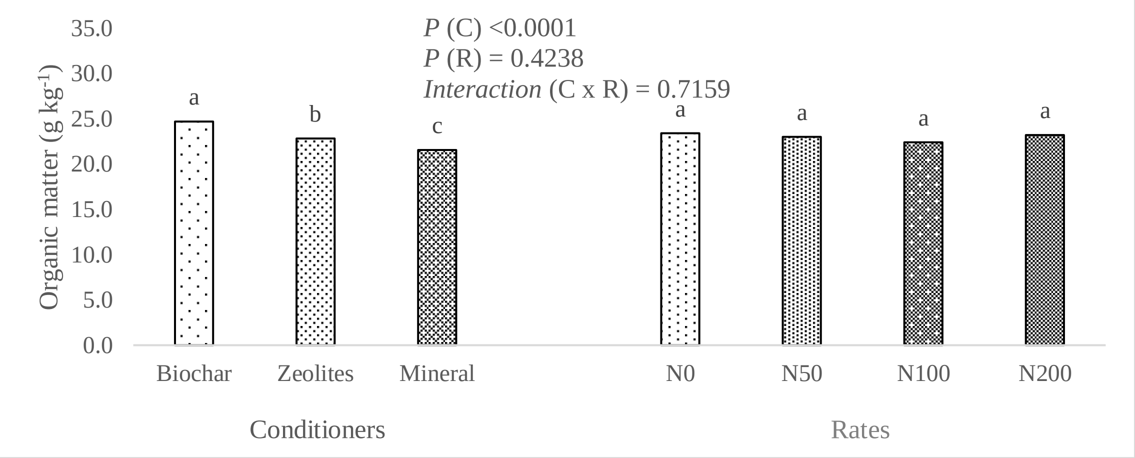


Figure 23: Soil organic matter in the field experiment as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.4.2. Soil pH

Soil pH values were determined in solutions of H_2O and KCl. There was found significant interaction between conditioners and nitrogen rates in pH (H2O), as well as significant differences among soil conditioners and among nitrogen rates (Figure 26). The use of Biochar significantly increased pH (5.80) over, Zeolites (5.70) and Mineral fertilizers (5.40). Soil pH decreased as the nitrogen rate increased. The values in the nitrogen treatments 0, 50, 100 and 200 kg N ha^{-1} were, respectively, 5.70, 5.69, 5.60 and 5.51.

There was also found significant interaction between soil conditioners and nitrogen rates in pH (KCl) (Figure 24). The values of pH (KCl) also significantly varied among soil conditioners but not among nitrogen rates. The soil conditioner with higher pH (KCl) was Biochar, with 5.20, followed by the Zeolites, with 4.90, and Mineral fertilizers, with 4.70.

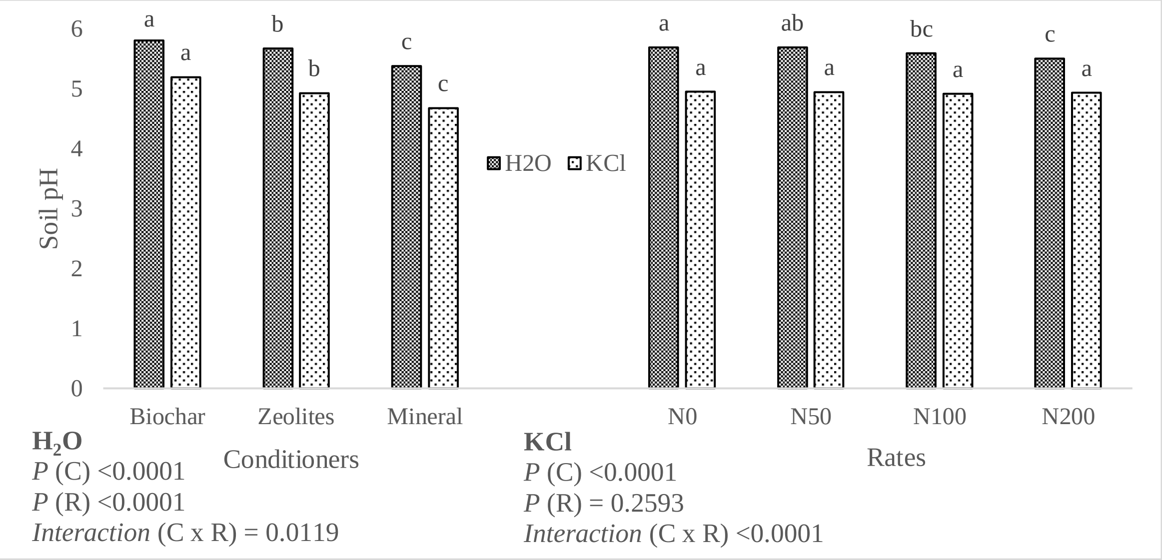


Figure 24: Soil pH measured in H_2O and KCl solutions at the end of the corn growing cycle in the field experiment as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

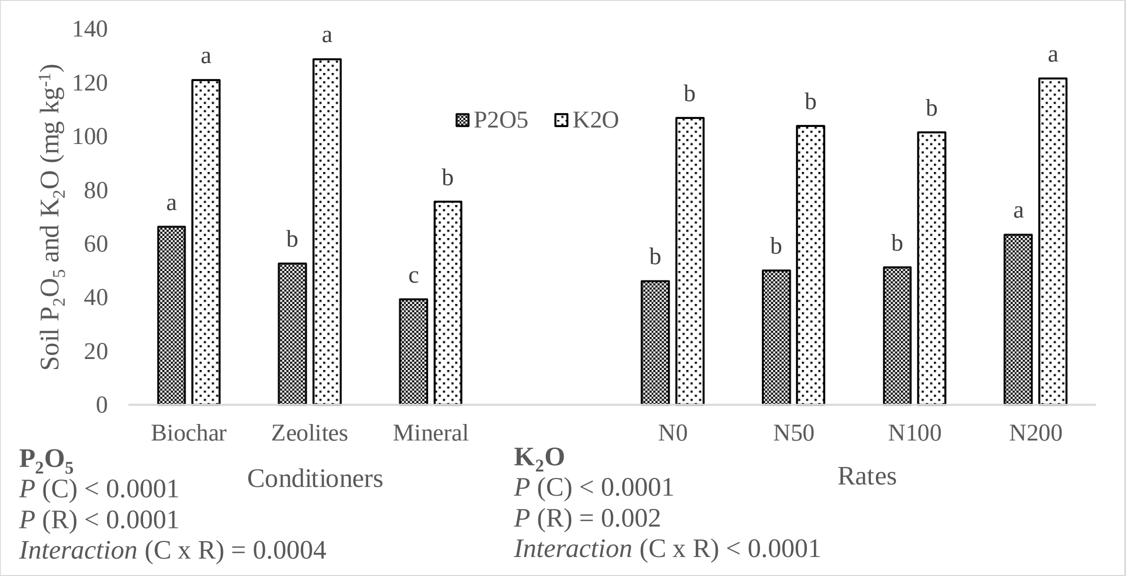
4.4.3. Soil phosphorus and potassium

Both soil phosphorus and potassium levels presented significant interaction between conditioners and nitrogen rates, likewise significant differences among these factors (Figure 25).

For the phosphorus concentration in the soil, Biochar was the conditioner with higher average value (66.3 mg P_2O_5 kg^{-1}), and Mineral fertilizers the one with lower average value, with 39.2 mg P_2O_5 kg^{-1}. As the nitrogen rate increased (0, 50, 100 and 200 kg N ha^{-1}, soil phosphorus levels increased as well (46.1, 50.1, 51.2 and 63.4 mg P_2O_5 kg^{-1}, respectively).

Among soil conditioners, the higher soil potassium levels were found in the Zeolites treatment, with 128.8 mg K_2O kg^{-1} and the lower in the Mineral fertilizers, with

75.7 mg K_2O kg^{-1}. For the different nitrogen rates, soil potassium levels had its higher average value in the treatment 200 kg N ha^{-1} (121.7 mg K_2O kg^{-1}) and the lower value in the treatment 100 kg N ha^{-1} (101.5 mg K_2O kg^{-1}).



P_2O_5
P (C) < 0.0001
P (R) < 0.0001
Interaction (C x R) = 0.0004

K_2O
P (C) < 0.0001
P (R) = 0.002
Interaction (C x R) < 0.0001

Figure 25: Soil phosphorus and potassium levels in the field experiment as a function of soil conditioner (C) and nitrogen rate (R). For each nutrient, the same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.4.4. Soil boron

Soil boron levels presented significant differences among the soil conditioners and nitrogen rates, but no significant interaction was found between the two factors (Figure 26). Zeolites was the soil conditioner showing higher average value (0.78 mg kg^{-1}), followed by Biochar (0.71 mg kg^{-1}) and Mineral fertilizers (0.48 mg kg^{-1}). As for the nitrogen rates, 0 kg N ha^{-1} was the treatment with higher average boron concentration, with 0.76 mg kg^{-1}, and the nitrogen treatment that presented lower soil boron levels was 100 kg N ha^{-1}, with 0.59 mg kg^{-1}.

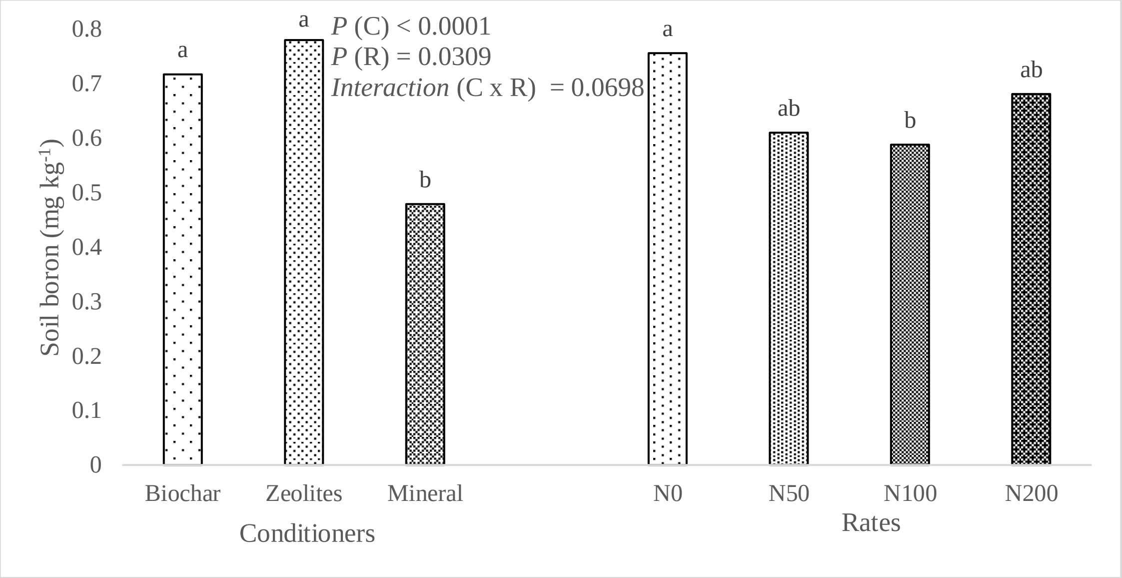


Figure 26: Soil boron concentration in the field experiment, as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.4.5. Exchange complex

Calcium and magnesium were the most concentrated cations of the exchange complex in the soil (Table 13). Both cations showed significant differences among soil conditioners and nitrogen rates, as well as significant interactions between the two factors. Biochar treated soils showed higher calcium and magnesium concentrations (12.36 cmol(+) kg^{-1} and 8.12 cmol(+) kg^{-1}, respectively) and the Mineral fertilizers the lower values (10.51 cmol(+) kg^{-1} and 5.75 cmol(+) kg^{-1}, respectively).

Among nitrogen rates, 0 kg N ha^{-1} had the higher concentrations of the cations, with 11.94 cmol(+) kg^{-1} of calcium and 7.36 cmol(+) kg^{-1} of magnesium. The lower concentration of calcium was found in the 200 kg N ha^{-1}, with 10.92 cmol(+) kg^{-1}, and the lower magnesium concentration in the 100 kg N ha^{-1}, with 6.52 cmol(+) kg^{-1}.

Exchange acidity did not present significant differences among the nitrogen rates, nor significant interaction between the two factors, however, the soil conditioners showed significantly differences in exchange acidity. Biochar was the conditioner with higher exchange acidity, reaching 0.34 cmol(+) kg^{-1}, and Mineral fertilizers the one with lower values (0.17 cmol(+) kg^{-1}).

There was found significant interaction in cation exchange capacity between soil conditioners and nitrogen rates, as well as significant differences among soil conditioners and among nitrogen rates. Biochar was the soil conditioner with the higher average CEC (22.16 cmol(+) kg^{-1}) and Mineral fertilizers the one with lower CEC (16.89 cmol(+) kg^{-1}). As for the different nitrogen rates, as the rate increased (0, 50, 100 and 200 kg N ha^{-1}), CEC values decreased (20.68, 19.65, 19.03 and 18.99 cmol(+) kg^{-1}, respectively).

Table 13: Calcium (Ca^{++}), magnesium (Mg^{++}), potassium (K^{+}), sodium (Na^{+}), exchange acidity (EA), aluminum (Al^{3+}) and effective cation exchange capacity (CECe) in soils from the field experiment as a function of soil conditioners (C) and nitrogen rates (R).

	Ca^{++}	**Mg^{++}**	**K^{+}**	**Na^{+}**	**EA**	**Al^{3+}**	**CECe**
	------------------------------------Cmol(+) kg $^{-1}$------------------------------------						
Biochar	12.36 a*	8.12 a	0.39 b	0.96 a	0.34 a	0.14 a	22.16 a
Zeolites	11.02 b	6.89 b	0.48 a	0.99 a	0.33 a	0.17 a	19.70 b
Mineral	10.51 b	5.75 c	0.24 c	0.22 b	0.17 b	0.09 b	16.89 c
N0	11.94 a	7.36 a	0.35 ab	0.69 a	0.32 a	0.14 a	20.68 a
N50	11.12 ab	7.17 a	0.40 ab	0.73 a	0.24 a	0.10 a	19.65 ab
N100	11.21 ab	6.52 b	0.31 b	0.73 a	0.27 a	0.08 a	19.03 b
N200	10.92 b	6.62 b	0.41 a	0.74 a	0.30 a	0.11 a	18.99 b
***P* (Conditioner)**	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0005	<0.0001
***P* (Rate)**	0.0385	<0.0001	0.0244	0.0552	0.2726	0.516	0.0021
***Interaction* (C x R)**	0.048	<0.0001	0.002	0.687	0.8606	0.802	0.0006

*The same letter in the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.5. NITROGEN USE EFFICIENCY

4.5.1. Stalk nitrate test

Stalk nitrate test was conducted in stalks from corn plants in the end of the growing cycle in the field experiment, as shown in Figure 27. There were found significant differences among the soil conditioners and among nitrogen rates, along with a significant interaction between these two factors.

Zeolites was the soil conditioner with higher nitrate concentration in stalks, reaching 6770 mg kg^{-1}, and Biochar presented lower values, with 2881 mg kg^{-1}. As for the different nitrogen rates, 200 kg N ha^{-1} outstand among all nitrogen treatments, with an average of 9648 mg kg^{-1}, and 0 kg N ha^{-1} presented the lower value, with 1544 mg kg^{-1}.

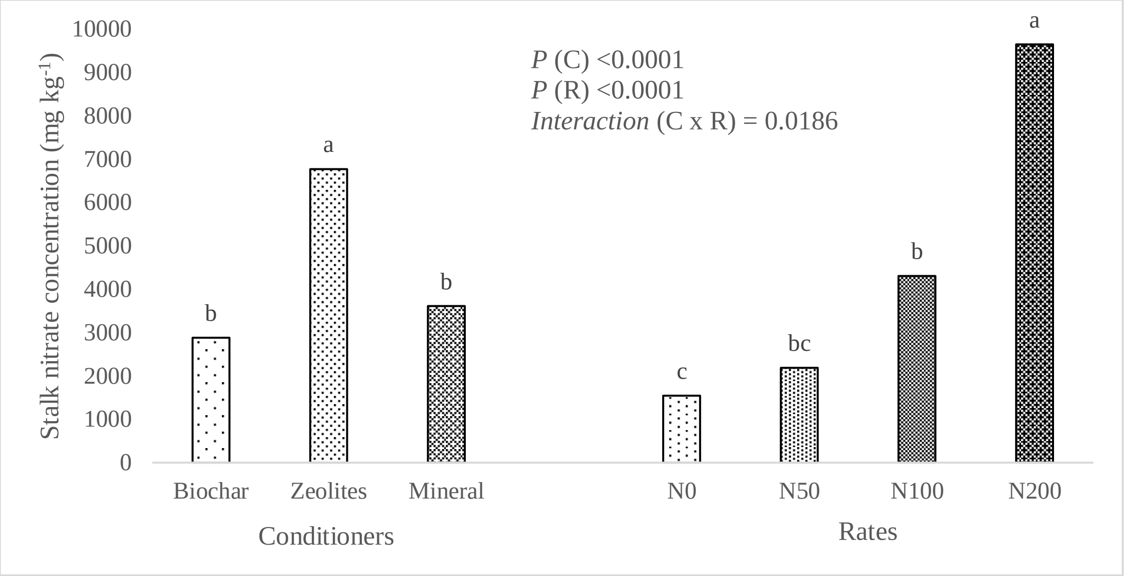


Figure 27: Stalk nitrate concentration in corn on the field experiment, as a function of soil conditioners (C) and nitrogen rates (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.5.2. Soil inorganic nitrogen at the end of the growing season

Soil nitrate concentration was measured a second time in the end of the growing season. The soil nitrate levels presented significant differences among soil conditioners and nitrogen rates, and no significant interaction was found between the two factors (Figure 28).

The higher soil nitrate levels were found in the plots treated with Biochar, which reached 129.9 mg kg^{-1}. It was followed by Zeolites, with 124.7 mg kg^{-1} and, with the lower soil nitrate concentration the Mineral fertilizers treatment with 112.7 mg kg^{-1}. For the different nitrogen treatments, as the nitrogen rate increased (0, 50, 100 and 200 kg N ha^{-1}), the soil nitrate concentration increased as well (80.2, 93.7, 128.5 and 185.9 mg kg^{-1}, respectively).

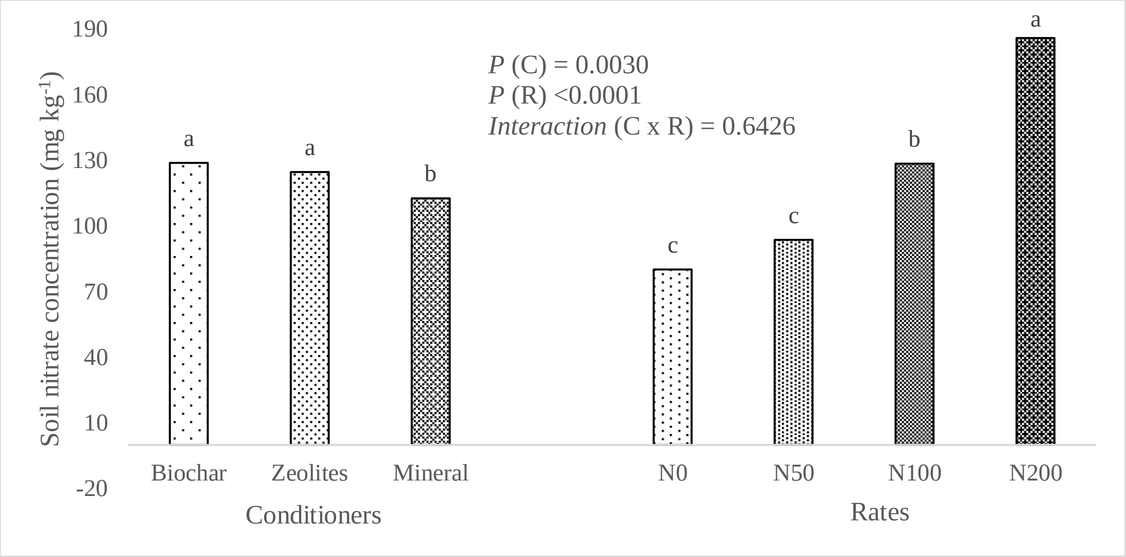


Figure 28: Soil nitrate concentration in the end of the corn growing season in the field experiment as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.5.3. Ammonium extracted by hot and cold KCl

Ammonium ion in the soil was extracted by cold and hot KCl. The values of NH_4^+ extracted by cold and hot KCl were presented in Figure 29. There were not found significant interactions for hot and cold KCl between soil conditioners and nitrogen rates. However, the values of NH_4^+ extracted by hot KCl significantly varied among soil conditioners and among nitrogen rates. Mineral fertilizer was the treatment showing higher NH_4^+ concentration (27.8 mg kg^{-1}) and Zeolites the lower concentration (18.5 mg kg^{-1}). As for the different nitrogen rates, as they increased (0, 50, 100 and 200 kg N ha^{-1}), NH_4^+ concentration also increased (20.2, 20.7, 21.9 and 25.1 mg kg^{-1}, respectively).

The values of NH_4^+ extracted by cold KCl also significantly varied among soil conditioners and nitrogen rates. Among the soil conditioners, Zeolites showed higher NH_4^+ concentration, with 8.9 mg kg^{-1}, and Mineral fertilizers the lower concentration, with 3.7 mg kg^{-1}. The different nitrogen rates had their higher NH_4^+ concentration in the 200 kg N ha^{-1} (11.1 mg kg^{-1}), and the lower in the 50 kg N ha^{-1} (4.4 mg kg^{-1}).

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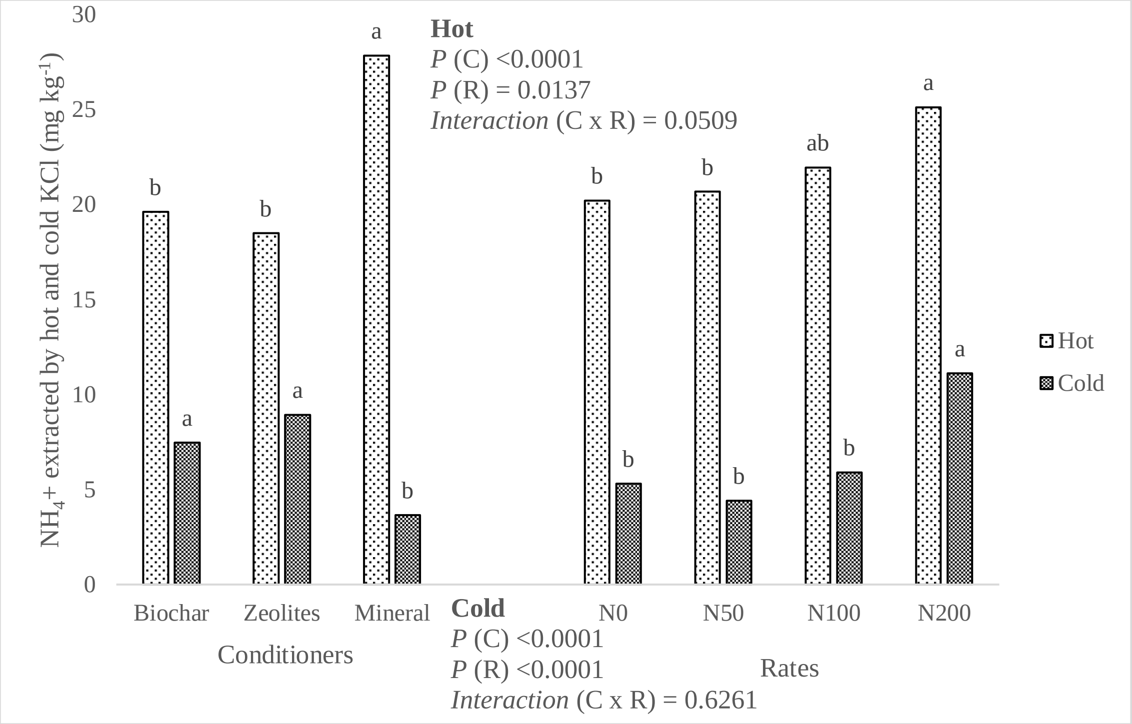

Figure 29: Ammonium extracted by hot and cold KCl in soils of the the field experiment as a function of soil conditioner (C) and nitrogen rates (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test (α = 0.05).

4.5.3.1. Hydrolysable nitrogen

Hydrolysable nitrogen was calculated as the subtraction of the NH_4^+ concentrations in the hot KCl extracts from the cold KCl extracts.

The Hydrolysable nitrogen presented significant differences among the soil conditioners, and significant interaction between soil conditioners and nitrogen rates (Figure 30). No significant differences were found among nitrogen rates.

Mineral fertilizers presented the higher values of hydrolysable nitrogen, with 24.1 mg kg^{-1}, followed by Biochar, with 12.1 mg kg^{-1} and Zeolites, with 9.5 mg kg^{-1}.

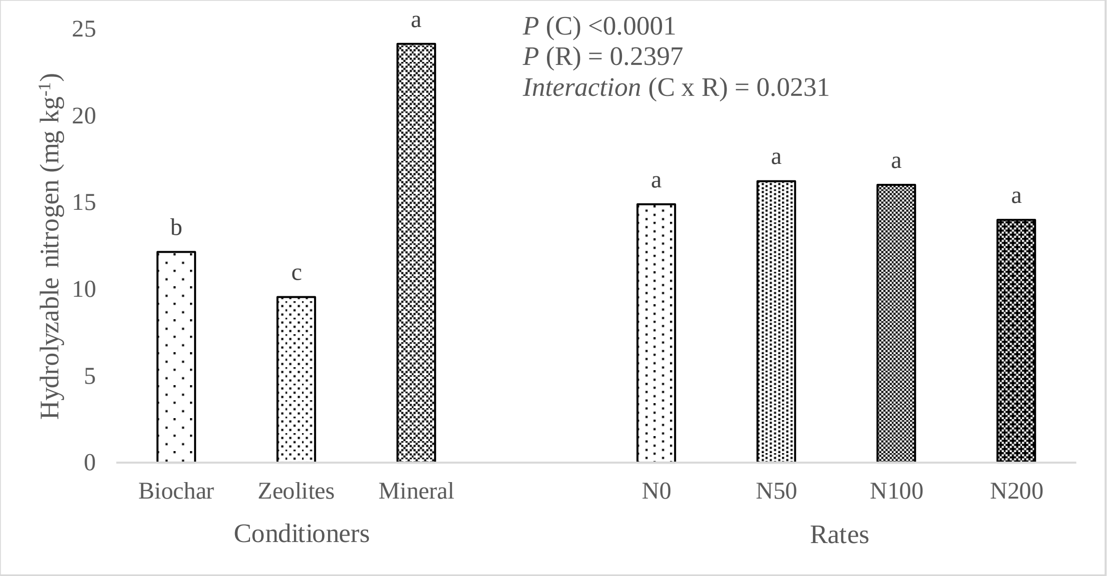

Figure 30: Hydrolysable nitrogen in the end of the corn growing season in the field experiment as a function of soil conditioner (C) and nitrogen rate (R). The same letter above the columns indicate the absence of significant differences between the treatments by the Tukey HSD test ($\alpha = 0.05$).

4.5.4. Apparent nitrogen recovery

Apparent nitrogen recovery was calculated for the field experiment and the pot experiment. In the field experiment, nitrogen recovery was measured for the whole plant collected in the end of the growing cycle, and the values found can be seen in Figure 31.

Biochar was the treatment with higher values. The plants that received Biochar plus 50 kg N ha^{-1} presented the highest value, with 142% of apparent nitrogen recovery, followed by the treatment Biochar plus 100 kg N ha^{-1}, with 97%, and Biochar plus 200 kg of N ha^{-1}, with 57%. For the Zeolites treatment, the higher value was found in the 50 kg N ha^{-1}, with 62% of apparent nitrogen recovery, the lower value with Zeolites was found in the 100 kg N ha^{-1} treatment, with 55%. Mineral fertilizers had their higher value in the 50 kg N ha^{-1} treatment, with 48% of apparent nitrogen recovery and the lower value of all the treatments in the 100 kg N ha^{-1} treatment, with -5%.

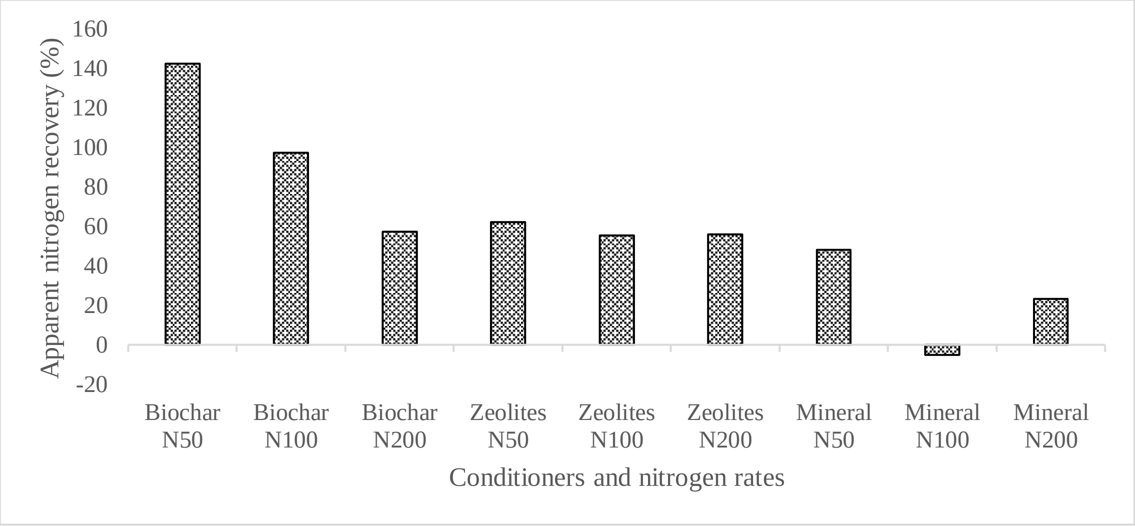

Figure 31: Apparent nitrogen recovery (ANR) in corn plants for different conditioners and rates.

In the pot experiment, the higher value was found in the Zeolites + N treatment, with 33% of apparent nitrogen recovery. The treatment that presented the lower apparent nitrogen recovery is the Nitrogen used alone used alone treatment, with 31% of apparent nitrogen recovery. This data can be seen in Figure 32.

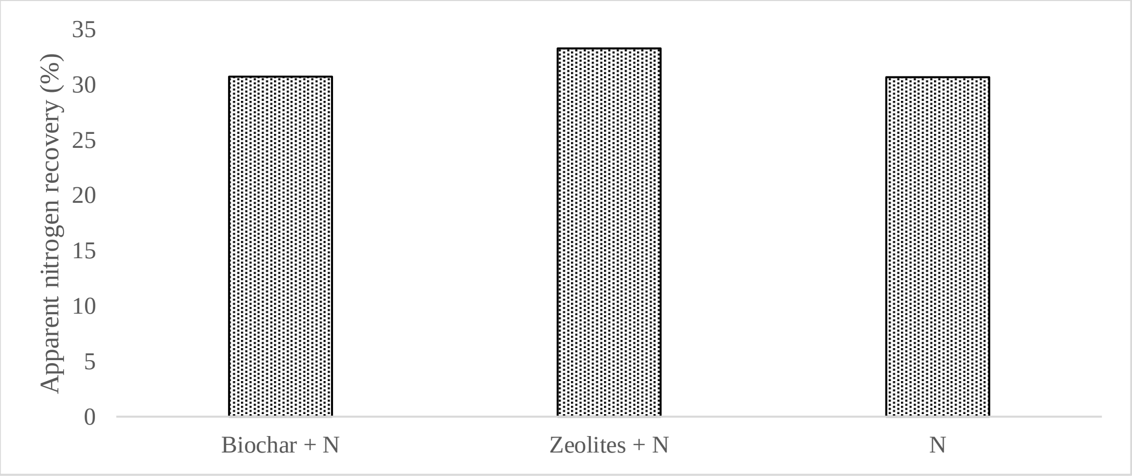

Figure 32: Apparent nitrogen recovery (ANR) in corn pot experiment.

5. DISCUSSION

The presence of a more controlled environment (pot experiment) showed different results to a typical crop area. It was observed that the different soil conditioners improve, in both experiments, on plant nutritional status and yield. However, each experiment had their particularities in the different analysis made.

5.1.FIELD EXPERIMENT

The pre-sidedress soil nitrate test showed the nitrate maximum average values in the Mineral fertilizers, with 91.6 mg kg^{-1}. This value is under the total soil nitrate parameter, issued by Portugal's Ministry of Agriculture and the Sea [84]. In the Biochar and Zeolites plots, nitrate concentration in the soil at pre-sidedress were found within the optimal nitrate range (10 – 50 mg kg^{-1})[83] with 34.0 and 36.7 mg kg^{-1}, respectively. This soil nitrate nitrogen index reveal good sensitivity to nitrogen fertilization as shown in previous studies [85].

Due to the negative charge of the anion nitrate, it moves freely in the soil solution, not being trapped on solid surfaces. Thus, it is noted that nitrate concentrations, at pre-sidedres, in the plots with the soil conditioners are lower than that in the plots with mineral fertilizers. These results suggest that Biochar and Zeolites may retain more nitrogen in the ammoniacal form, contributing to the nitrate concentration reduction and minimizing potential risks of leaching [86], [87].

However, when analysed soil ammonium at pre-sidedress, all treatments were within the optimal NH_4^+ range (0 – 5 mg kg^{-1})[83], and the Mineral fertilizers demonstrated the higher ammonium concentration of 2.6 mg kg^{-1}, followed by the Zeolites, with 2.5 mg kg^{-1} and Biochar with 2.0 mg kg^{-1}. Therefore, the soil conditioners did not demonstrate the higher ammoniacal nitrogen retention at pre-sidedress, as expected. Probably some biological immobilization had occurred as a stimulus of soil conditioners in biological activity.

NDVI meter and the fluorometer were not able to discriminate between the nutritional status of the plants in the different treatments. However, for the SPAD reading, Mineral fertilizers and Zeolites showed higher results (respectively, 59.0 and 58.0). Argenta [88] analyzed SPAD readings in different corn hybrids and nitrogen rates and, as the nitrogen rate increased, SPAD values increased as well. The same pattern happened in the field experiment, when its higher readings were in 200 kg N ha^{-1} (62.0). Argenta's study also shows that these values are directly correlated with plant's nutritional status.

To better characterize plant's condition in mid-cycle, leaf analysis were made and, as expected, when compared to the SPAD readings the plots containing Mineral fertilizers demonstrated higher potassium, calcium and magnesium concentration, and the ones with Zeolites the higher nitrogen, phosphorus and micronutrients concentration in corn leaves.

In the end of the growing season, dry matter yield was determined, and the higher values were found for the Zeolites (15.7 t ha^{-1}). A study made by Covalsky [11] showed that Zeolites provide nutrients in greater amounts to plants gradually over time, increasing plant yield and enhancing their nutritional balance, especially when it comes to nitrogen and phosphorus concentration.

At the end of the growing season, whole corn plant nutritional conditions expose the Zeolites effect on final nitrogen concentration. The Zeolites showed the higher nitrogen concentration of 12.6 g kg^{-1}, as well as higher phosphorus and calcium concentration. Biochar increases potassium concentration [89], therefore this soil conditioner presents the higher potassium concentration in corn plants at the end of the growing season, with 13.1 g kg^{-1}. Biochar also demonstrated high values for plant's micronutrients, specially boron and iron.

Pointing out to the soils, Biochar was the soil conditioner with greater results. These results show the influence of Biochar application on soil organic matter content. In fact, the introduction of Biochar in the soil increases its carbon content, contributing to the higher organic matter value (25 g kg^{-1}). The results may be influenced by the physical and chemical properties of Biochar, such as the high-density charge, resulting in a high nutrients retention and their natural combination with particles with specific chemical structure. These factors form complexes that are more resistant to microbial degradation than other organic matter in the soil [90].

In soil pH, it was already expected that the high pH value of Biochar (Table 2) would contribute to a higher pH value of the soils where it was applied, yet the pH was not as neutral or basic as expected, reaching 5.8. Agegnehu [91] reported increases in pH from 7.1 to 8.1, due to the addition of Biochar, but there are still works where the addition of Biochar has little influence on the pH increase, when related to soils of high cation exchange capacity.

Phosphorus had its higher concentration in the Biochar plots, reaching 66.3 mg P_2O_5 kg^{-1}. Apparently, Biochar allowed this nutrient to remain more available in the soil solution. The surface charges retained cations available in the soil, allowing the phosphate ion to remain available. An increase in soil available phosphorus due to the use of biochar is a result frequently reported by other researchers [92][86].

For potassium concentration, the Zeolites plots presented the higher values (128.8 mg K_2O kg^{-1}). In general, Zeolites are associated with higher soil potassium concentrations. This fact may be related to the potassium concentration in this soil conditioner (Table 2) which represents an additional dosage of this nutrient.

In Portugal, the values of cation exchange capacity (CEC) parameters are often low, less than 10 cmol(+) kg^{-1} due to the predominantly acidic nature of the soils [93]. For this study, the higher CEC value was found in Biochar, with 22.16 cmol(+) kg^{-1}. This factor may be due to the calcium and magnesium concentration and the exchange acidity, that had their higher values in the Biochar plots, with, respectively, 12.36, 8.12 and 0.34 cmol(+) kg^{-1}.

The field experiment was conducted in order to quantify and characterize nitrogen use-efficiency.

Stalk nitrate test evaluates the adequacy of nitrogen application during the growing season. According to Brouder [94], the optimum nitrate concentration is from 450 to 2000 mg kg^{-1}, this value would allow the yield to not be limited by nitrogen. However, this study has shown values above 2000 mg kg^{-1} in all soil conditioners. The treatments with 0 kg N ha^{-1} are the only ones with an average nitrate concentration in between the recommended concentration, with 1544 mg kg^{-1}.

The apparent nitrogen recovery showed that Biochar with 50 kg N ha^{-1} have the higher nitrogen recovery, with 142%. Studies show that the use of Biochar and Zeolites help in the nitrogen concentration and recovery [95].

For the soil inorganic nitrogen concentration from the end of the crop cycle, the maximum expected concentration, according to the USDA [96], from soils collected between July and November, is between 100 and 150 mg kg^{-1}. The higher inorganic nitrogen concentration was found in the Biochar plots, with 129.9 mg kg^{-1}. This factor delegitimizes the fact that Biochar retains soil nitrogen in the ammoniacal form and contributes to nitrate concentration reduction.

However, the soil ammonium concentration in the end of the crop cycle from the cold KCl solution, emphasizes that Zeolites retains soil nitrogen in the ammoniacal form. This soil conditioner presents the higher ammonium values, with 8.93 mg kg^{-1}.

The ammonium retention in the hot KCl solution presents different results, showing that Mineral fertilizers have higher ammonium concentration (27.85 mg kg^{-1}). The hot KCl solution evaluates labile fractions of soil organic matter and brings improvements in the estimation of N availability for the crop, therefore, the hydrolysable nitrogen results match with this evaluation, showing that Mineral fertilizers presented 24.15 mg kg^{-1} of hydrolysable nitrogen concentration.

The interaction found in most of the tests demonstrated the correlation between the conditioners applied and the different nitrogen rates and how they influence on plant nutritional status and yield.

5.2. POT EXPERIMENT

In the pot experiment, the chlorophyll response tests in July did not present any significant differences among the treatments, except for the SPAD readings, that showed the Zeolites + N better chlorophyll response, with an index of 39. However, in August, SPAD, NDVI and fluorometer readings presented significant differences among treatments that confirm Zeolites + N better chlorophyll response and green color intensity. Zeolites + N better response may be since this conditioner provides, gradually, greater nutrient amounts, increasing plant yield and enhancing their nutritional balance [11].

As expected, nitrogen concentration in plant tissues in the pot experiment, had its higher values in the treatments with an extra nitrogen dosage, however, among them, the Nitrogen used alone presented higher nitrogen concentration, with 20.3 g kg^{-1}. It was expected that the Zeolites + N or Biochar + N presented higher nitrogen concentration, because these soil conditioners are supposed to increase and retain plant nitrogen and help on phosphorus and potassium concentration [95]. Phosphorus and potassium did not present any significant differences among the treatments.

The Zeolites used alone presented higher calcium concentration, with 3.04 g kg^{-1} and Biochar + N had the higher values for magnesium, with 3.61 g kg^{-1}. These results show that the soil conditioners increase the nutrients concentration in plant tissues.

For the micronutrients, the Nitrogen used alone treatment presented the higher average values, specially for zinc and manganese. Nitrogen fertilization enhances micronutrients concentration and helps on plant health [6].

Plant height's first measurement, in July, showed that the Biochar + N plants were higher, reaching 0.86 m, but in August, the Zeolites + N treatment plants had a growth spurt and reached 1.17 m. The Zeolites' affinity for nutrients allows this mineral to be used in organic substrates to stimulate crop growth. This factor shows that, even in early stages, the soil conditioners influence on plant structure and the nitrogen dosage came as an assistance to maintain these results.

Even though in the end of the crop cycle the Zeolites + N plants were higher, Biochar + N demonstrated greater dry matter yield (51 g plant^{-1}). Regarding Biochar, the response and use of it in the agricultural crops varied according to the characteristics of the soil. Crops have been performing well when applying Biochar to the soil [95].

Apparent nitrogen recovery shows that the Zeolites + N treatment recovered 33% of nitrogen. Studies show that Zeolites application increased corn yield in 43.5% in unfertilized plants and 3.4% in fertilized plants in relation to the control treatment, thus demonstrating a higher nitrogen uptake by the plants[12].

6. CONCLUSIONS AND FUTURE WORKS

6.1. CONCLUSIONS

For both, field and pot experiment, the use of soil conditioners demonstrated better results in the soil-plant environment.

Plant production, in the field experiment, demonstrated the Zeolites' treatments aboveground biomass increase, as well as a better nutritional status, especially in nitrogen concentration. Biochar presented higher apparent nitrogen recovery, showing its potential nutritional balance improvement in following growing seasons.

Soil properties at harvest demonstrate Biochar efficiency and nutritional improvement during the growing season. The different nitrogen rates influenced on soil properties as well, especially in the 200 kg N ha^{-1}, however, its higher dosage may cause future problems in nitrate and ammonium concentration, and, therefore inducing leaching risks.

In the pot experiment, the treatments that received an extra nitrogen dosage outstand from the ones that did not, showing that the nitrogen fertilization, combined with the soil conditioners can help on plant development. The Zeolites + N treatment was the one with better plant nutritional improvement and growth and Biochar + N treatment demonstrated a significant increase in the aboveground biomass.

6.2. FUTURE WORKS

For the field experiment the following research are suggested, considering that not all of them should be placed in the same area of this work, because of the soil conditioners impact in the following growing crops after the first year.

- Biochar, Zeolites and Mineral fertilizers response to different nitrogen rates in the second growing season;
- Impact of the summer Biochar and Zeolites fertilization in winter crops soil and dry matter yield;
- The influence of increasing Biochar and Zeolites rates, coupled up with increasing and decreasing nitrogen rates in the soil-plant environment;

- Evaluation of Biochar and Zeolites influence in corn ear size and nutritional balance;
- The use of satellite imagery, aero-photography and field sampling (SPAD, NDVI and Fluorometer) in plant development, when exposed to different soil conditioners and nitrogen rates, during the growing season and in the following years.

Even though the pot experiment has more controlled environment, some suggestions are presented for future research and continuation of the previous work.

Before installing the experiment 48 sacks were sewed and then filled with Biochar and Zeolites. The sacks were from canvas, so the product could be in touch with the pots' soil and the plants' roots. There were four treatments that had the sacks, and each treatment had three sacks per pot. The soil and sacks were kept in the greenhouse area to future evaluation.

- Observe the sacks impact in soil-plant holding, use and exchange of nutrients capacity in the same pots for the following growing seasons;
- Evaluate root growth and development after the use of Biochar and Zeolites, with and without an extra nitrogen dosage;
- Combine Biochar and Zeolites as a treatment and evaluate if, together, they influence positively on soil-plant environment;
- The influence of increasing Biochar and Zeolites rates, coupled up with increasing and decreasing nitrogen rates in soil-plant development in a controlled environment;
- Evaluation of Biochar and Zeolites influence in corn ear size and nutritional balance in a controlled environment.

REFERENCES

[1] A. M. Coelho, “Nutrição e Adubação do Milho,” 2006.

[2] M. A. G. Cuenca, C. C. Nazário, D. C. Mandarino, and M. G. L. Manos, “Aspectos da Cultura do Milho: Características e Evolução da Cultura no Estado do Maranhão entre 1990 e 2003,” Aracaju, 2005.

[3] D. Beegle, “Nitrogen Fertilization of Corn,” no. 3, pp. 1–13, 2013.

[4] D. Lapola *et al.*, “Mudanças Ambientais Globais - Abertura,” 2017.

[5] L. de O. Machado, “Adubação Nitrogenada.” [Online]. Available: https://www.scribd.com/document/118365773/Apostila-Adub-Nitrogenada-02. [Accessed: 14-Dec-2018].

[6] K. G. Cassman, A. Dobermann, and D. T. Walters, “Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management,” *AMBIO A J. Hum. Environ.*, vol. 31, no. 2, pp. 132–140, Mar. 2002.

[7] B. Basso, B. Dumont, D. Cammarano, A. Pezzuolo, F. Marinello, and L. Sartori, “Environmental and economic benefits of variable rate nitrogen fertilization in a nitrate vulnerable zone,” *Sci. Total Environ.*, vol. 545–546, pp. 227–235, Mar. 2016.

[8] M. Â. Rodrigues and J. F. Coutinho, *Eficiência de utilização do azoto pelas plantas*. .

[9] G. C. Pontalti, “NITRITOS E NITRATOS: VENENOS OU NUTRIENTES?,” 2011.

[10] D. L. Silva, “EFEITO DA APLICAÇÃO DE CONDICIONADORES DO SOLO NA BIODISPONIBILIDADE DE METAIS PESADOS,” 2018.

[11] C. D. A. Covalski, “Influência da aplicação de Biochar e Zeólitos na dinâmica do azoto no sistema solo-planta,” 2018.

[12] E. Carvalho Brasil and L. Cunha Da Hungria, “Aproveitamento de nitrogênio por plantas de milho em resposta à adubação nitrogenada a base de zeólita enriquecida com uréia.”

[13] J. L. da S. Nunes, “Características do Milho,” *2016*. [Online]. Available: https://www.agrolink.com.br/culturas/milho/informacoes/caracteristicas_361401.html. [Accessed: 14-Dec-2018].

[14] Anpromis, “O Milho,” 2018. [Online]. Available: http://www.anpromis.pt/o-milho.html. [Accessed: 12-Dec-2018].

[15] P. C. Magalhães and F. O. M. Durães, “Fisiologia da Produção de Milho,” 2006.

[16] P. C. Mangelsdorf, “Corn. Its origin, evolution and improvement.,” *Corn. Its Orig. Evol. Improv.*, no. 2. ed., 1974.

[17] G. W. Beadle, “The ancestry of corn.,” *Sci. Am.*, vol. 242, pp. 112–119, 1980.

[18] J. Doebley, A. Stec, J. Wendelt, and M. Edwardst, “Genetic and morphological

analysis of a maize-teosinte F2 population: Implications for the origin of maize (molecular markers/evolution/Zea/restriction fragment length polymorphism/quantitative genetics),” 1990.

[19] H. Wang *et al.*, “The origin of the naked grains of maize,” *Nature*, vol. 436, no. 7051, pp. 714–719, Aug. 2005.

[20] J. C. Garcia, M. J. Mattoso, J. de O. Duarte, and J. C. Cruz, “Aspectos Econômicos da Produção e Utilização do Milho,” 2006.

[21] T. L. F. Guth, “Panorama do Milho,” 2018.

[22] USDA, “World Agricultural Production,” 2018.

[23] I. Formigoni, “Compare a evolução na produção de milho no mundo, por país produtor,” 2016. [Online]. Available: http://www.farmnews.com.br/mercado/producao-de-milho-no-mundo/. [Accessed: 11-Dec-2018].

[24] Eurostat, *Agriculture, forestry and fishery statistics 2017 edition*. 2017.

[25] Agrotec, “Área de milho aumenta em Portugal,” 2018. [Online]. Available: http://www.agrotec.pt/noticias/area-de-milho-aumenta-em-portugal/. [Accessed: 11-Dec-2018].

[26] Anpromis, “Evolução das áreas de Milho - Total Nacional (Grão/ Silagem) 2004 a 2018,” 2018.

[27] L. de V. Souza, *A produção de milho em Portugal*, no. 2. Publindústria, 2011.

[28] P. C. Magalhães and F. Durães, “Fisiologia da Produção de Milho,” 2006.

[29] G. G. de Sousa, C. F. de Lacerda, L. F. Cavalcante, F. V A Guimarães, M. E. de J Bezerra, and G. L. da Silva, “Nutrição mineral e extração de nutrientes de planta de milho irrigada com água salina 1,” *Rev. Bras. Eng. Agrícola e Ambient.*, vol. 14, no. 11, pp. 1143–1151, 2010.

[30] D. L. Broch and S. K. Ranno, “Fertilidade do solo, adubação e nutrição da cultura do milho,” in *Tecnologia e Produção: Soja e Milho*, 2012, pp. 240–252.

[31] A. Olness and G. R. Benoit, “A Closer Look at Corn Nutrient Demand.”

[32] S. C. S. Cruz, F. R. Da, S. Pereira, J. R. Santos, A. W. De Albuquerque, and E. T. Da Silva, “Parcelamento da adubação nitrogenada na cultura do milho irrigado em sistema plantio direto,” 2008.

[33] N. N. Greenwood and A. Earnshaw, “Chemistry of the elements,” *Cryst. Res. Technol.*, vol. 20, no. 5, pp. 662–662, May 1985.

[34] K. Barbalace, “Periodic Table of Elements: Nitrogen - N,” 2018. [Online]. Available: https://environmentalchemistry.com/yogi/periodic/N.html. [Accessed: 14-Dec-2018].

[35] D. R. Williams, “Earth Fact Sheet,” 2017. [Online]. Available: https://nssdc.gsfc.nasa.gov/planetary/factsheet/earthfact.html. [Accessed: 14-Dec-2018].

[36] B. Z. Houlton, S. L. Morford, and R. A. Dahlgren, “Convergent evidence for

widespread rock nitrogen sources in Earth’s surface environment,” *Science (80-.).*, vol. 360, no. 6384, pp. 58–62, Apr. 2018.

[37] R. F. Vieira, *Ciclo do nitrogênio em sistemas agrícolas*. 2017.

[38] “The 4 Steps of NITROGEN CYCLE: The Key to the Food Chain - Earth How.” [Online]. Available: https://earthhow.com/nitrogen-cycle/. [Accessed: 14-Dec-2018].

[39] L. B. Mendes, I. de F. F. Tinôco, C. de F. Souza, and J. A. O. Saraz, “O ciclo do nitrogênio na criação de frangos de corte e suas perdas na forma de amônia volátil: uma revisão,” pp. 10–12, 2012.

[40] A. F. Campos and E. N. de Lima, *Ciclo do Nitrogênio: Abordagem em livros didáticos de ciências do ensino fundamental*, vol. 13, no. 1. UFRGS, 1996.

[41] Q. Xiao, L.-X. Zhu, H.-P. Zhang, X.-Y. Li, Y.-F. Shen, and S.-Q. Li, “Soil amendment with biochar increases maize yields in a semi-arid region by improving soil quality and root growth,” *Crop Pasture Sci.*, vol. 67, no. 5, p. 495, 2016.

[42] B. R. Cohen, “The Story of N: A Social History of the Nitrogen Cycle and the Challenge of Sustainability,” *Agric. Hist.*, vol. 89, no. 1, pp. 117–118, 2015.

[43] D. Fowler *et al.*, “Effects of global change during the 21st century on the nitrogen cycle,” *Atmos. Chem. Phys.*, vol. 15, no. 24, pp. 13849–13893, Dec. 2015.

[44] C. Qiao, L. Liu, S. Hu, J. E. Compton, T. L. Greaver, and Q. Li, “How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input,” *Glob. Chang. Biol.*, vol. 21, no. 3, pp. 1249–1257, Mar. 2015.

[45] S. Niu *et al.*, “Global patterns and substrate-based mechanisms of the terrestrial nitrogen cycle,” *Ecol. Lett.*, vol. 19, no. 6, pp. 697–709, Jun. 2016.

[46] A. C. Andrade, D. M. da Fonseca, D. S. Queiroz, L. T. Salgado, and P. R. Cecon, “Adubação nitrogenada e potássica em capim-elefante (Pennisetum purpureum Schum. cv. Napier),” *Ciência e Agrotecnologia*, pp. 1643–1651, 2003.

[47] M. C. J. Belarmino, J. C. Pinto, G. P. Rocha, A. E. Furtini Neto, and A. R. de Morais, “Altura de perfilho e rendimento de matéria seca de capim-tanzânia em função de diferentes doses de superfosfato simples e sulfato de amônio,” *Ciência e Agrotecnologia*, vol. 27, no. 4, pp. 879–885, Aug. 2003.

[48] S. Deuner, R. do Nascimento, L. S. Ferreira, P. G. Badinelli, and R. S. Kerber, “Adubação foliar e via solo de nitrogênio em plantas de milho em fase inicial de desenvolvimento,” *Ciência e Agrotecnologia*, vol. 32, no. 5, pp. 1359–1365, Oct. 2008.

[49] W. A. R. Lara Cabezas, B. J. R. Alves, S. S. U. Caballero, and D. G. de Santana, “Influência da cultura antecessora e da adubação nitrogenada na produtividade de milho em sistema plantio direto e solo preparado,” *Ciência Rural*, vol. 34, no. 4, pp. 1005–1013, Aug. 2004.

[50] A. K. Alva, S. Paramasivam, A. Fares, J. A. Delgado, D. Mattos, and K. Sajwan, “Nitrogen and Irrigation Management Practices to Improve Nitrogen Uptake Efficiency and Minimize Leaching Losses,” *J. Crop Improv.*, vol. 15, no. 2, pp.

369–420, Jun. 2006.

[51] K. G. Cassman, G. C. Gines, M. A. Dizon, M. I. Samson, and J. M. Alcantara, “Nitrogen-use efficiency in tropical lowland rice systems: contributions from indigenous and applied nitrogen,” *F. Crop. Res.*, vol. 47, no. 1, pp. 1–12, Jul. 1996.

[52] R. D. Hauck and J. E. Harper, “Uptake of Organic Nitrogen Forms by Roots and Leaves,” in *Nitrogen in Crop Production*, vol. acsesspublicati, no. nitrogenincropp, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 1984, pp. 165–170.

[53] M. Z. Copperi, M. E. Mandolesi, M. A. Cantamutto, and M. M. Ron, “Comparative effectiveness of two nitrogen sources for corn fertilization Efectividad comparativa de dos fuentes nitrogenadas para la fertilización del maíz,” *Phyton-International J. Exp. Bot.*, vol. 82, pp. 99–106, 2013.

[54] L. dos S. lucas *et al.*, “Ação de organismos diazotróficos sob fungos micorrízicos arbusculares em rizosfera de mucuna preta.,” *Congr. Interdiscip. - ISSN 2595-7732*, 2017.

[55] W. J. O. Souza and W. J. Melo, “TEORES DE NITROGÊNIO NO SOLO E NAS FRAÇÕES DA MATÉRIA ORGÂNICA,” 2000.

[56] L. Rodrigues De Oliveira, G. Vieira Miranda, R. Oliveira Delima, R. Fritsche-Neto, J. Carlos, and C. Galvão, “Eficiência na absorção e utilização de nitrogênio e atividade enzimática em genótipos de milho,” no. 3, pp. 614–621.

[57] A. G. Sal, “Aspectos ecológicos de los sistemas agrícolas. Las dimensiones del desarrollo.,” *Mundi Prensa*, pp. 83–119, 2001.

[58] S. J. Sarandón and C. C. Flores, *Agroecología: bases teóricas para el diseño y manejo de Agroecosistemas sustentables*, 1st ed. Edulp, 2014.

[59] P. Petersen, L. M. da Silveira, G. B. Fernandes, and S. G. Almeida, *MÉTODO DE ANÁLISE ECONÔMICO-ECOLÓGICA DE AGROECOSSISTEMAS*, 1st ed. Rio de Janeiro, 2017.

[60] A. Feiden, “Agroecologia: Introdução e Conceitos.”

[61] P. Montserrat, *La cultura que hace el paisaje : escritos de un naturalista sobre nuestros recursos de montaña*. 2009.

[62] P. H. Mayer, “FERTILIDADE DO SISTEMA AGRÍCOLA: ESTUDO EM TRÊS COMUNIDADES DA REGIÃO METROPOLITANA DE CURITIBA- PR,” 2009.

[63] A. M. Primavesi, A. Nobre, and S. Gliessman, “Agroecossistemas e a ecologia da vida do solo: Por uma outra forma de agricultura,” *IHU on-line*, vol. 485, 2016.

[64] P. K. R. Nair, R. Buresh, D. Mugendi, and C. Latt, “Nutrient Cycling in Tropical Agroforestry Systems: Myths and Science,” 1999.

[65] V. Smil, “Nitrogen cycle and world food production.”

[66] L. Alberto Navarro de Araújo, M. Evaristo Ferreira, and M. Cristina Pessôa da Cruz, “Adubação nitrogenada na cultura do milho.”

[67] R. G. M. Backer, W. Saeed, P. Seguin, and D. L. Smith, “Root traits and nitrogen

fertilizer recovery efficiency of corn grown in biochar-amended soil under greenhouse conditions," *Plant Soil*, vol. 415, no. 1–2, pp. 465–477, Jun. 2017.

[68] J. P. R. Do Amaral Filho, D. Fornasieri Filho, R. Farinelli, and J. C. Barbosa, "Espaçamento, densidade populacional e adubação nitrogenada na cultura do milho," *Rev. Bras. Cienc. do Solo*, vol. 29, no. 3, pp. 467–473, 2005.

[69] P. Mancus, "Nitrogen Fertilizer Dependency and Its Contradictions: A Theoretical Exploration of Social-Ecological Metabolism," *Rural Sociol.*, vol. 72, no. 2, pp. 269–288, Jun. 2007.

[70] International Fertilizer Association, "Nutrient Management Handbook," 2016.

[71] H. F. J. Reetz, *Fertilizers and their efficient use*. 2016.

[72] M. D. Taylor, "Accumulation of cadmium derived from fertilisers in New Zealand soils," *Sci. Total Environ.*, vol. 208, no. 1–2, pp. 123–126, Dec. 1997.

[73] J. L. Abranches, R. L. Ferreira, and M. J. Perdoná, "MITIGAÇÃO DA CONTAMINAÇÃO AMBIENTAL PELO USO DE UREIA REVESTIDA POR POLÍMEROS NA AGRICULTURA," vol. XII, 2016.

[74] M. Lemunier *et al.*, "Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts.," *Appl. Environ. Microbiol.*, vol. 71, no. 10, pp. 5779–86, Oct. 2005.

[75] P. Cintra, "EFEITO DE DIFERENTES TIPOS DE CONDICIONADORES DE SOLO, FERTILIZANTES E MANEJO DO MATO NA UMIDADE DO SOLO DA LAVOURA CAFEEIRA."

[76] Ministério da Agricultura Pecuária e Abastecimento, "INSTRUÇÃO NORMATIVA N 25, DE 23 DE JULHO DE 2009," 2009.

[77] Ministério da Agricultura Pecuária e Abastecimento, "INSTRUÇÃO NORMATIVA Nº 35, DE 4 DE JULHO DE 2006," 2006.

[78] A. L. F. Luz, "FERTILIZANTES, COBERTURAS E CONDICIONADORES DE SOLO NO CONTROLE DA CERCOSPORIOSE DO CAFEEIRO," Lavras, 2017.

[79] M. Laghari *et al.*, "Recent developments in biochar as an effective tool for agricultural soil management: a review," *J. Sci. Food Agric.*, vol. 96, no. 15, pp. 4840–4849, Dec. 2016.

[80] L. F. C. Leite, E. S. Mendonça, J. C. L. Neves, P. L. O. A. Machado, and J. C. C. Galvão, "ESTOQUES TOTAIS DE CARBONO ORGÂNICO E SEUS COMPARTIMENTOS EM ARGISSOLO SOB FLORESTA E SOB MILHO CULTIVADO COM ADUBAÇÃO MINERAL E ORGÂNICA," *Rev. Bras. Ciência do Solo*, vol. 27, pp. 821–832, 2003.

[81] "Google Earth © 2018 Google Image © 2019 Digital Globe," 2019. .

[82] PhysicsOpenLab, "NDVI Index," 2017. [Online]. Available: http://physicsopenlab.org/2017/01/30/ndvi-index/. [Accessed: 25-Feb-2019].

[83] D. A. Horneck, D. M. Sullivan, J. S. Owen, and J. M. Hart, "Soil test interpretation guide," *Oregon State Univ. Ext. Serv.*, no. July, pp. 1–12, 2011.

[84] Direção regional de Agricultura e Pescas de Lisboa e Vale do Tejo, "Caderno de

Campo da Zona Vulnerável a Nitratos – Tejo –,” 2012.

[85] M. Â. Rodrigues, “Establishment of continuous critical levels for indices of plant and presidedress soil nitrogen status in the potato crop,” *Commun. Soil Sci. Plant Anal.*, vol. 35, no. 13–14, pp. 2067–2085, 2004.

[86] S. Gao, T. H. DeLuca, and C. C. Cleveland, “Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: A meta-analysis,” *Sci. Total Environ.*, vol. 654, pp. 463–472, 2019.

[87] S. Li, S. Wang, and Z. Shangguan, “Combined biochar and nitrogen fertilization at appropriate rates could balance the leaching and availability of soil inorganic nitrogen,” *Agric. Ecosyst. Environ.*, vol. 276, pp. 21–30, 2019.

[88] G. Argenta, P. R. F Silva, E. L. Fosthofer, M. L. Strieder, E. Suhre, and L. L. Teichmann, “ADUBAÇÃO NITROGENADA EM MILHO PELO MONITORAMENTO DO NÍVEL DE NITROGÊNIO NA PLANTA POR MEIO DO CLOROFILÔMETRO,” 2003.

[89] C. C. MARTINS, “BIOCHAR, COMPOSTO ORGÂNICO E POTÁSSIO NAS CARACTERÍSTICAS QUÍMICAS E LIXIVIAÇÃO DE NUTRIENTES EM ESPODOSSOLO E NO CULTIVO DE MUCUNA PRETA E MORINGA,” 2018.

[90] I. P. C. Nóbrega, “Efeitos do Biochar nas propriedades físicas e químicas do solo,” Lisboa, 2011.

[91] G. Agegnehu, A. K. Srivastava, and M. I. Bird, “The role of biochar and biochar-compost in improving soil quality and crop performance: A review,” *Appl. Soil Ecol.*, vol. 119, pp. 156–170, Oct. 2017.

[92] S. Gul and J. K. Whalen, “Biochemical cycling of nitrogen and phosphorus in biochar-amended soils,” *Soil Biol. Biochem.*, vol. 103, pp. 1–15, Dec. 2016.

[93] LQARS, *Manual de Fertilização das Culturas*. 2006.

[94] S. M. Brouder, “Cornstalk Testing to Evaluate the Nitrogen Status of Mature Corn: Nitrogen Management Assurance,” 2003.

[95] A. Nair and V. Lawson, “Effect Of Biochar On Sweet Corn Production,” no. August, pp. 1–10, 2012.

[96] USDA, “Soil Nitrate,” *NRCS East Natl. Technol. Support Cent.*, no. 3, pp. 1–2, 2014.