University of Zagreb Faculty of Agriculture

Ghent University Faculty of Bioscience Engineering

Mihaela Šatvar Vrbančić

UTILIZATION OF NITROGEN FROM DIGESTATE AS A SUBSTITUTE FOR MINERAL NITROGEN FERTILIZERS

INTERNATIONAL DUAL DOCTORATE

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GHENT UNIVERSITY

Sveučilište u Zagrebu Agronomski fakultet

Sveučilište u Gentu, Fakultet biotehničkih znanosti

Mihaela Šatvar Vrbančić

ISKORISTIVOST DUŠIKA IZ DIGESTATA U ODNOSU NA MINERALNA DUŠIČNA GNOJIVA

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I, **Mihaela Šatvar Vrbančić**, hereby declare that I have completed solely myself the the thesis titled:

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With my signature, I confirm that:

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- I am familiar with the provisions of the Code of Ethics of the University of Zagreb (Article 19).

Zagreb, 2026

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Doctoral candidate's signature

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IZJAVA O IZVORNOSTI

Ja, **Mihaela Šatvar Vrbančić**, izjavljujem da sam samostalno izradila doktorski rad pod naslovom:

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Professor Čoga's research interests include plant nutrition, ecology and soil improvement. During his time at the Faculty of Agriculture, he took part in a number of scientific and professional projects. As a proof of his outstanding scientific and professional activity in the field of agronomy, Professor Dr. sc. Lepomir Čoga has published more than 128 scientific papers (as author or co-author) and presented them at conferences in Croatia and abroad, including 21 original scientific papers of group A1 (papers cited in the bibliographic databases Current Contents and SCI). He is an co-author of one book, author of internal script and reviewer of another book.

In addition to his scientific and professional work, Professor Dr. sc. Lepomir Čoga is particularly distinguished by his teaching activities at the Faculty of Agriculture of the University of Zagreb. He holds several modules within the Bologna Process, including one at the undergraduate level in Zagreb (Ishrana bilja u fitomedicini i Metode i dijagnostika u ishrani bilja). He also teaches modules at graduate level in Zagreb (Ishrana bilja u zaštićenim prostorima i Primjena fermentiranog ostatka nakon proizvodnje bioplina kao gnojiva) and a module in postgraduate doctoral studies in Agricultural Sciences (Uloga ishrane bilja u očuvanju okoliša). He also contributes to academic programs outside his home college and teaches at the University of Josip Juraj Strossmayer, Faculty of Agrobiotechnical Sciences in Osijek.

During his many years of teaching, Professor Dr. sc. Lepomir Čoga has supervised diploma, master's and doctoral theses and has been chairman or member of committees for many other scientific works.

He is a member of the Croatian Society of Soil Science.

Professor Čoga's contributions to the field are also evidenced by his extensive publications, with his research focusing on sustainable agricultural practices, bio-based fertilizers and the environmental impact of agricultural activities.

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https://www.agr.unizg.hr/hr/member/63

Biography (CROSBI): https://www.croris.hr/osobe/profil/11874

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Professor Dr. Erik Meers is a distinguished academic and researcher in the field of environmental technology and circular bioeconomy. He is currently a professor at the Department of Green Chemistry and Technology of the Faculty of Bioscience Engineering at Ghent University, Belgium. His expertise lies in the fields of nutrient recovery, waste recycling and sustainable agriculture

Professor Meers obtained his PhD in 2005 in the Department of Biosciences and Engineering, specialising in soil remediation through phytomanagement. After his PhD, he conducted postdoctoral research in the field of environmental remediation in collaboration with industry. Between 2007 and 2012, he worked as National Business & Technology Developer for a multinational energy producer, specialised in biogas projects and related research and development. From 2012 to 2016 he coordinated the regional biogas platform in Flanders, Belgium (Biogas-E). In 2016, he returned to Ghent University where he set up a bioresource recovery team that now includes 50 employees.

His research includes the recovery of nutrients from manure and digestate from biomethanization, the recycling of technology-critical elements from waste and wastewater, and the development of micronutrient-enriched soil fertilisers. He has coordinated several major scientific projects, including studies on the application of compost from municipal solid waste in agriculture and the use of bioenergy crops for phytoremediation of metal-enriched agricultural soils.

Professor Meers has founded eight small and medium-sized enterprises and established platforms for science communication, business development and community, including Biorefine Cluster Europe, Re-Source.Bio and NutriCycle Vlaanderen.

He is actively involved in various scientific networks, including the European Biogas Association (EBA), where he serves as Chairman of the Scientific Advisory Board, and the European Sustainable Phosphorus Platform (ESPP), where he is a member of the Steering Committee.

Professor Meers has an extensive publication list with over 380 publications and more than 16 000 citations reflecting his significant contributions to the field.

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"Per aspera ad astra"

ABSTRACT

The move towards sustainable agriculture requires greater use of bio-based fertilizers (BBFs) such as digestate, the nutrient-rich by-product of anaerobic digestion (AD). Digestate is increasingly recognized as a valuable organic fertilizer due to its high content of key macronutrients such as nitrogen (N), phosphorus (P) and potassium (K). Despite its agricultural potential, the widespread use of digestate is still limited by problems such as variability in chemical composition, high application and storage costs and regulatory requirements. The effectiveness of digestate can vary depending on the feedstock source (animal and plant), soil type and environmental conditions, making standardized application difficult. Nevertheless, previous studies have shown that digestate can be successfully used as an alternative nutrient source in a number of agricultural fields.

The aim of this study was to evaluate the effects of applying digestate fractions (solid and liquid) and conventional mineral N fertilizers (NPK and CAN) on crop performance, soil properties and the potential for nitrate leaching in acidic soils in two consecutive growing seasons (2018 and 2019). Digestate fractions were obtained by anaerobic co-digestion of maize silage and liquid cattle manure. Seven treatments were applied to maize crops in four replicates: unfertilized control (C), mineral fertilizer (NPK+CAN), liquid cattle manure (LCM), solid fraction (SFD), liquid fraction of digestate (LFD) and combinations of SFD and LFD with mineral fertilizer (with 50% of total N supplied from digestate fractions and 50% from mineral fertilizer). In 2018, significantly higher yields were achieved on average compared to 2019. In average compared to the control treatment (7.9 t ha^{-1}), the statistically significant highest grain yields were achieved with the treatments NPK+CAN (12.1 t ha^{-1}), LFD+NPK (11.8 t ha^{-1}) and SFD+NPK (11.0 t ha^{-1}). A significantly higher N fertilizer recovery value (NFRV) was recorded in both years for the treatments NPK+CAN (100 %) and LFD+NPK (80.5 %).

The results confirm that, when properly managed, digestate is a reliable source of plant-available N comparable to conventional mineral fertilizers. Despite environmental variations, nitrate concentrations in the soil (including possible leaching) remained within safe limits and no negative effects on plant health were observed, neither from nitrate accumulation nor from heavy metal concentrations. Furthermore, the results show that combining the LFD+NPK achieved the highest yield results, demonstrating the potential to reduce mineral fertilizer use by up to half while contributing to lower greenhouse gas emissions and promoting healthier, more resilient soils. In addition, this fertilization strategy can improve nutrient use efficiency, support long-term soil fertility by increasing organic matter content, and provide economic benefits to farmers by reducing production costs while minimizing the environmental risks associated with intensive mineral fertilization.

Overall, these results highlight the role of digestate in promoting sustainable and economically viable nutrient management strategies, while providing valuable data to support the development of future regulatory guidelines and standardization of digestate application on agricultural land in Croatia.

Key words: anaerobic digestion, digestate fractions; nitrogen fertilization; maize grain yield; nitrate leaching; heavy metal; soil pH

SAŽETAK

Održiva poljoprivreda zahtijeva što manju upotrebu mineralnih gnojiva te prijelaz na upotrebu gnojiva na bazi biomase odnosno proizvode iz organskih materijala prirodnog podrijetla. Jedan od njih je i digestat, prirodni bogati nusproizvod anaerobne digestije (AD). Digestat je sve više prepoznat kao vrijedno organsko gnojivo zbog visokog sadržaja važnih makrohraniva kao što su dušik (N), fosfor (P) i kalij (K). Unatoč njegovom agronomskom potencijalu, šira primjena digestata i dalje je ograničena zbog problema poput varijabilnosti kemijskog sastava digestata, visokih troškova primjene i skladištenja, te zakonodavstva. Učinkovitost digestata može varirati ovisno o vrsti sirovine koja ulazi u AD (životinjskog ili biljnog podrijetla), tipu tla i okolišnim uvjetima, što otežava standardiziranu primjenu. Dosadašnja istraživanja pokazala su da se digestat može uspješno koristiti kao alternativni izvor hraniva u različitim poljoprivrednim sustavima.

Cilj ovog istraživanja bio je ispitati učinke primjene frakcija digestata (krute i tekuće) i konvencionalnih mineralnih gnojiva sa N (NPK i CAN) na prinos kukuruza, svojstva tla i potencijal ispiranja nitrata na kiselom tlu tijekom dvije uzastopne vegetacijske sezone (2018. i 2019.). Frakcije digestata dobivene su anaerobnom kodigestijom silaže kukuruza i tekućeg goveđeg gnoja. Prije i nakon sjetve kukuruza primijenjeno je 7 različitih tretmana u 4 ponavljanja: kontrolni tretman bez gnojidbe (C), mineralno gnojivo (NPK+CAN), tekući goveđi gnoj (LCM), kruta frakcija digestata (SFD), tekuća frakcija digestata (LFD) te kombinacije SFD ili LFD s mineralnim gnojivom (pri čemu je 50 % ukupnog dušika osigurano iz frakcija digestata, a 50 % iz mineralnog gnojiva). Značajno veći prinosi prosječno su zabilježeni u 2018 godini u odnosu na 2019. U usporedbi s kontrolnim tretmanom (7,9 t ha^{-1}), statistički značajni najveći prinos zrna zabilježen je na tretmanu NPK+CAN (12,1 t ha^{-1}), LFD+NPK (11,8 t ha^{-1}) i SFD+NPK (11,0 t ha^{-1}). Značajno veća iskoristivost N iz gnojiva (NFRV) bila je utvrđena na tretmanima NPK+KAN (100 %) i LFD+NPK (80,5 %) kroz obje godine.

Dobiveni rezultati ovog istraživanja potvrđuju da je digestat, kada se pravilno primjenjuje, pouzdan izvor biljci dostupnog N, usporediv s konvencionalnim mineralnim dušičnim gnojivima. Unatoč okolišnim uvjetima, koncentracije nitrata u tlu (uključujući moguće ispiranje) ostale su unutar dopuštenih granica, a negativni učinci na zdravlje biljaka nisu zabilježeni, ni nakupljanjem nitrata ni koncentracijom teških metala. Nadalje, rezultati pokazuju da LFD+NPK tretman postiže najbolje rezultate u prinosu, ukazujući na mogućnost smanjenja uporabe mineralnih gnojiva, što rezultira smanjenju emisije stakleničkih plinova i promicanje zdravijih i otpornijih tala. Osim toga, ovakva strategija gnojidbe može poboljšati učinkovitost korištenja hraniva, dugoročno pridonijeti plodnosti tla povećanjem sadržaja organske tvari te pružiti ekonomsku korist poljoprivrednicima smanjenjem troškova proizvodnje, uz smanjenje ekoloških rizika povezanih s intenzivnom mineralnom gnojidbom.

Sveukupno, ovi rezultati naglašavaju važnu ulogu digestata u promicanju održivih i ekonomski isplativih strategija upravljanja hranivima te daju vrijedne podatke za buduće zakonodavne smjernice i standardizaciju primjene digestata na poljoprivrednim površinama u Hrvatskoj.

Ključne riječi: anaerobna digestija; frakcije digestata; gnojidba dušikom; prinos zrna kukuruza; ispiranje nitrata; teški metali; pH tla

SAMENVATTING (Extended summary in Dutch):

De overgang naar duurzame landbouw vereist een grotere inzet van biogebaseerde meststoffen (BBM's), zoals digestaat, het nutriëntenrijke bijproduct van anaerobe vergisting (AV). Digestaat wordt steeds meer erkend als een waardevolle organische meststof vanwege het hoge gehalte aan essentiële macronutriënten zoals stikstof (N), fosfor (P) en kalium (K). Ondanks het agronomische potentieel wordt het wijdverbreide gebruik van digestaat beperkt door problemen zoals variabiliteit in chemische samenstelling, hoge toepassings- en opslagkosten en regelgeving. De effectiviteit van digestaat kan variëren afhankelijk van de bron van het substraat (dierlijk of plantaardig), het bodemtype en de omgevingsomstandigheden, wat standaardisatie van de toepassing bemoeilijkt. Niettemin hebben eerdere studies aangetoond dat digestaat met succes kan worden gebruikt als alternatieve nutriëntenbron in verschillende landbouwsectoren.

Het doel van deze studie was om het effect te bepalen van bemesting met digestaatfracties (vast en vloeibaar) als gedeeltelijke of volledige vervanging van minerale meststoffen (NPK en CAN) op de opbrengst en kwaliteit van maïs, de beschikbaarheid van stikstof over tijd, evenals de mate van verontreiniging van het "bodem-plant" systeem met schadelijke anorganische verontreinigingen (zware metalen en nitraten). Het experiment werd uitgevoerd op zure bodems gedurende twee opeenvolgende groeiseizoenen (2018 en 2019). De digestaatfracties werden verkregen door anaerobe co-vergisting van maïssilage en vloeibare rundermest van de biogasinstallatie Bojana in Čazma, Kroatië. Zeven behandelingen werden toegepast op maïsgewassen in vier herhalingen: onbehandelde controle (C), minerale meststof (met 50% van de totale N uit NPK en 50% uit CAN – NPK+CAN), vloeibare rundermest (LCM), vaste fractie (SFD), vloeibare fractie van digestaat (LFD) en combinaties van SFD en LFD met minerale meststof (met 50% van de totale N geleverd uit digestaatfracties en 50% uit minerale meststof). De toepassingsdosering werd vastgesteld op 140 kg totale N in alle bemestingsbehandelingen. Aangezien het experimentele veld valt onder de Nitraatgevoelige Zones (NVZ), werd een dosering van 140 kg N ha^{-1} toegepast om nitraatuitspoeling na de oogst te voorkomen, hoewel de Nitraatrichtlijn 170 kg totale N ha^{-1} toestaat. Bodem- en plantmonsters werden genomen tijdens drie vegetatieve fasen van maïs: de vegetatieve fase V4 (vier volledig ontwikkelde bladeren), de reproductieve fase R5 (silomaïs, dent stadium) en de reproductieve fase R5 (fysiologische rijpheid van maïs). Vervolgens werden de bodem- en plantmonsters geanalyseerd in het laboratorium van de Afdeling Plantenvoeding, Faculteit Landbouw, Zagreb, Kroatië, en in het laboratorium van de Faculteit Bio-ingenieurswetenschappen van de Universiteit Gent in België.

In 2018 werden gemiddeld significant hogere opbrengsten behaald in vergelijking met 2019. Ten opzichte van de controlebehandeling (7,9 t ha^{-1}) werden de hoogste statistisch significante graanopbrengsten behaald met de behandelingen NPK+CAN (12,1 t ha^{-1}), LFD+NPK (11,8 t ha^{-1}) en SFD+NPK (11,0 t ha^{-1}). In hetzelfde jaar werden de hoogste ANR (Apparente Stikstofterugwinning) waarden waargenomen in de NPK+CAN en LFD+NPK behandelingen. Deze twee behandelingen bereikten ook de hoogste NFRV (Stikstofmeststofvervangingswaarde). Een vergelijkbare trend werd waargenomen in 2019, waarbij NPK+CAN, SFD+NPK en LFD+NPK de hoogste ANR-waarden hadden. Statistisch gezien leverden NPK+CAN en LFD+NPK consequent de

hoogste NFRV-waarden. Na NPK+CAN (100±0) had LFD+NPK de op één na hoogste NFRV-waarde met 83±9.

Wat betreft nitraatuitspoeling werden in 2018 statistisch significante verschillen gevonden tussen de behandelingen, waarbij NPK+CAN en LFD+NPK de hoogste NO_3^-–N-residuen in de bodem na de oogst hadden. De resultaten suggereren dat het gebruik van SFD en LFD het risico op nitraatresidu-accumulatie of uitspoeling niet verhoogt in vergelijking met NPK+CAN. Dit geeft aan dat bemesting met digestaat geen negatieve effecten heeft op de bodem of planten door verhoogde nitraatuitspoeling. Bovendien tonen de resultaten aan dat het milieu niet wordt geschaad, aangezien de kleine hoeveelheden uitgeloogde nitraten op een bodemdiepte van 0–90 cm verwaarloosbaar waren.

Daarnaast bleven de concentraties van zware metalen (Ni, Pb, Co, Cr, Cd) in de bodem en in de maïsplanten onder de kritieke waarden bij gebruik van zowel de vaste als vloeibare digestaatfracties. Er kan dus worden geconcludeerd dat digestaat van de Bojana-biogasinstallatie geschikt is voor landbouwgebruik en niet bijdraagt aan de accumulatie van zware metalen in de bodem of planten.

De resultaten bevestigen dat digestaat, mits goed beheerd, een betrouwbare bron van plantbeschikbare stikstof is, vergelijkbaar met conventionele minerale meststoffen. Ondanks variaties in omgevingsomstandigheden bleven de nitraatconcentraties in de bodem (inclusief mogelijke uitspoeling) binnen veilige grenzen en werden geen negatieve effecten op de plantgezondheid waargenomen, noch door nitraataccumulatie, noch door zware metaalconcentraties. Bovendien tonen de resultaten aan dat het combineren van de vloeibare fractie van digestaat met minerale meststoffen de hoogste opbrengstresultaten opleverde, wat het potentieel aantoont om het gebruik van minerale meststoffen tot de helft te verminderen, terwijl tegelijkertijd wordt bijgedragen aan lagere broeikasgasemissies en gezondere, veerkrachtigere bodems worden bevorderd. Daarnaast kan deze bemestingsstrategie de nutriëntenefficiëntie verbeteren, de langetermijnbodemvruchtbaarheid ondersteunen door het verhogen van het organische stofgehalte, en economische voordelen bieden aan boeren door de productiekosten te verlagen en tegelijkertijd de milieurisico's die gepaard gaan met intensieve minerale bemesting te minimaliseren.

Over het geheel genomen bevestigden de resultaten al onze drie onderzoekshypothesen en onderstreepten ze het potentieel van digestaat om duurzame en economisch haalbare nutriëntenbeheerstrategieën te ondersteunen. Deze studie levert ook waardevolle gegevens voor de ontwikkeling van toekomstige wettelijke richtlijnen en standaardisatie van digestaattoepassing op landbouwgrond in Kroatië. In het bijzonder werd bevestigd dat de combinatie van minerale meststoffen met de vloeibare fractie van digestaat de beste resultaten oplevert wat betreft maïsopbrengst en -kwaliteit, terwijl gedeeltelijke vervanging van minerale stikstof mogelijk is. Bovendien werd bevestigd dat stikstofmineralisatie en -vrijgave uit digestaat in de loop van de tijd toenemen. Ten slotte bevestigden de resultaten dat bemesting met digestaat geen risico vormt voor de verontreiniging van de bodem of planten met nitraten of zware metalen.

Trefwoorden: anaerobe vergisting, digestaatfracties, stikstofbemesting, maïs graanopbrengst, nitraatuitspoeling, zware metalen, bodem-pH

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LIST OF ABBREVIATIONS

AD	Anaerobic digestion
N	Nitrogen
P	Phosphorous
K	Potassium
EU	European Union
NH_4^+	Ammonia
NO	Nitric oxide
N_2O	Nitrous oxide
CO_2	Carbon dioxide
H_2	Hydrogen
BBFs	Bio-based fertilizers
NMIT	Nitrogen mineralization and immobilization
C/N	Nitrogen to carbon ratio
NO_3^-	Nitrate
NUE	Nitrogen use efficiency
LFD	Liquid fraction of digestate
SFD	Solid fraction of digestate
ANR	Apparent nitrogen recovery
NFRV	Nitrogen fertilizer replacement value
RES	Renewable energy source
HORTE	Croatian energy market operator
C	Unfertilizerd control
NPK+CAN	Mineral fertilizer NPK 15-15-15 (50%) + CAN 27%N (50%)
LCM	Liquid cattle manure
SFD+NPK	Mixture of mineral fertilizer (50%) with solid fraction of digestate (50%)
LFD+NPK	Mixture of mineral fertilizer (50%) with liquid fraction of digestate (50%)
NVZ	Nitrate voulnerable zones
DHMZ	Croatian meteorological and hydrological station
APMP	The annual cource of the amount of precipitation (mm)
S	Sulfur

Zn	Zinc
V4	Vegetative stage with four fully developed leaves
R5	Reproductive dent stage or stage of maize sillage
R6	Reproductive stage or physiological maturity
DM	Dry matter
OM	Organic matter
OC	Organic carbon
EC	Conductivity
pH	Soil reaction
ON	Organic nitrogen
Ca	Calcium
Mg	Magnesium
Cu	Copper
ANOVA	Analysis of variance
PVC	Poly vinyl chloride
WFPS	Water filled pore space
Nrel,net	Nitrogen release
Nrel,min	Nitrogen mineralization
TSMN	Total soil mineral nitrogen
FW	Fresh weight
DW	Dry weight
TDGY	Total dry grain yield
GLM	General linear model

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1. INTRODUCTION

Digestate, the nutrient-rich by-product of anaerobic digestion (AD) of materials such as manure (cattle, pig, poultry, etc.), crop residues and food waste, is increasingly recognized as a valuable organic fertilizer (Wallace, 2011; Alburquerque et al., 2012; Möller & Müller, 2012; Sigurnjak et al., 2017; Chojnacka and Moustakas, 2024). It is particularly rich in important macronutrients such as nitrogen (N), phosphorus (P) and potassium (K), as well as micronutrients. However, its use is associated with certain logistical and financial challenges, including high costs for application and storage (Saedi et al., 2008; Đurđević et al., 2018; Kovačić et al., 2022). The chemical composition of digestate varies depending on feedstock and processing conditions, and its effectiveness can differ depending on plant species and soil type, making standardized use complex (Đurđević et al., 2018; Kovačić et al., 2022). While this variability can make widespread application difficult, studies by Vágó et al. (2009) and Möller et al. (2008) have shown that digestate can serve as an effective nutrient source in a range of soil and environmental conditions, as supported by additional studies (Makádi et al., 2012; Doyeni et al., 2021; Czekala, 2022).

During the anaerobic digestion (AD) process, complex organic compounds are broken down into simpler forms, leaving a large proportion of nutrients in the final digestate. Nitrogen is converted to a considerable extent, with a significant proportion being converted to the ammonium form (NH_4^+), which is readily available to plants (Wallace, 2011; Nkoa, 2014). Depending on the type of feedstock used, the availability of N in digestate can vary, typically around 50% for cattle slurry, 70% for pig slurry and up to 80% for food digestate in the first year of application (WRAP, 2012; WRAP, 2016; WOOD, 2020). In contrast to mineral fertilizers, digestates provide residual N release over a longer period of time, as the decomposition of organic matter can extend over more than one year. This characteristic gives digestate a potential advantage over other organic fertilizers in terms of sustainable soil health and fertility (Lund & Doss, 1980; Magdoff and Amadon, 1980; Görlitz et al., 1985; Werner et al., 1985; Sommerfeldt et al., 1988; Dilz et al., 1990; Wysocka-Czubaszek, 2019).

The biogas sector in the European Union (EU) has grown significantly — from around 6 277 plants in 2009 to nearly 19 491 biogas plants (plus ~1 324 biomethane facilities) by 2022, according to the 2023 EBA Statistical Report. This growth has varied across the continent, with new markets being developed in Eastern Europe and existing infrastructure being expanded in Western Europe (EBA, 2017). Despite the progress made in biogas energy production, digestate is still underutilized and often treated as a waste product (Weiland, 2010; Đurđević et al., 2020). Germany is the EU leader in digestate production with around 87 million tons per year, followed by countries such as Italy, the United Kingdom and France (WOOD, 2020). These countries generally also have robust incentive structures that support biogas as a renewable energy source (EBA, 2018; WOOD, 2020).

In Croatia, the development of biogas has so far been more modest, but there has been rapid progress in recent years. In 2009, there were only three biogas plants, of which only one used agricultural raw materials for energy production. There are 42 operating plants (FZOEU, 2024) with a total capacity of almost 39 MW although most still rely heavily on maize silage and manure rather than waste streams (EBA, 2018; Đurđević et al., 2018). Commonly used feedstocks include cattle and pig manure (50–60%), silage (25–35%) and other biodegradable materials such as food waste and sewage sludge (Đurđević et al., 2018).

About 60% of the N in animal-based digestate is in the form of ammonia (NH_4^+) (WRAP, 2012; WRAP, 2016; Sigurnjak et al., 2017), which is highly available to plants but can also leach into groundwater if not treated properly (Biró et al., 2005; Alburquerque et al., 2012). Therefore, the use of digestate is regulated by the Nitrates Directive (91/676/EEC), which limits N application to 170 kg N ha^{-1} and promotes practices that protect water from nutrient pollution. In Croatia, regulatory oversight is currently based on the Ordinance on the Protection of Agricultural Land from Pollution (NN 71/19). While there is no specific framework for the certification or marketing of digestate, a new regulation is currently being drafted and a public consultation on the draft regulation on Croatian Fertilizing Products is underway. In this draft, digestate is recognized and classified as a soil improver.

In addition to its nutritional value, the use of digestate also contributes to climate change mitigation. It contributes to the reduction of greenhouse gas emissions by replacing mineral fertilizers and storing carbon in the soil (Möller et al., 2008). However, despite its agronomic, economic and environmental potential, digestate still faces obstacles such as quality, limited awareness among farmers, knowledge and the lack of uniform legislation (Šatvar Vrbančić et al., 2025). This research aims to help close these gaps by providing data that will assist in the valorization of digestate as a viable substitute for mineral fertilizers. It also aims to inform potential investors and stakeholders about the economic and environmental value of digestate in order to contribute to more sustainable agricultural practices. Ultimately, one of the biggest challenges in Croatia remains the knowledge gap among farmers and the lack of a clear policy at both national and EU level. Addressing these challenges could unlock the full potential of digestate and make it an essential part of the transition toward more sustainable agriculture.

1.1. OBJECTIVE AND HYPOTHESES OF RESEARCH

The hypotheses put forward in this dissertation were as follows:

I. The combination of mineral fertilizer with the liquid fraction of digestate will result with the best yield and quality of maize, and will replace part of the nitrogen from mineral fertilizer.

II. Mineralization and release of N from digestate will increase with time interval from treatment.

III. Digestate fertilization will not have a harmful effect on the soil and the plant.

Based on the hypotheses, the following research objectives were defined:

I. To determine the effect of digestate fertilization as a partial or complete replacement of mineral fertilizers on the yield and quality of maize.

II. Determine the availability of nitrogen with a time interval of treatment.

III. Determine the degree of contamination of the "soil - plant" system with harmful inorganic pollutants (heavy metals and nitrates).

2. LITERATURE REVIEW

2.1. GLOBAL USE OF MINERAL AND ORGANIC FERILIZERS

As the world's population grows, so does the demand for food, water and energy. It is estimated that 9.7 billion people will live on Earth in 2050 (FAO, 2022). Meeting this demand is a major challenge, especially given the increasing dependence on finite resources and the environmental impacts associated with intensive agriculture. To address these issues, the European Commission has launched initiatives such as the Circular Economy Action Plan (European Commission, 2014–2020), which aims to promote smarter use of resources and reduce waste in all sectors — with agriculture playing a central role. Healthy soils are the foundation of sustainable food systems (FAO, 2023), and understanding soil fertility allows farmers to apply fertilizers more precisely, resulting in higher yields, lower greenhouse gas emissions and minimized nutrient losses (Wang et al., 2018; Javed et al., 2022).

Traditionally, synthetic mineral N fertilizers, developed using the Haber–Bosch process, have played a crucial role in securing the world's food supply (Gilland, 2014; Kuriyama & Hayashi, 2025). However, this method is energy-intensive and heavily dependent on fossil fuels, which contributes significantly to greenhouse gas emissions. Even more worrying is the inefficiency of current N use in agriculture: only about half of applied is actually taken up by the plants, the rest is lost through leaching, volatilization and crop residues (Leip et al., 2014; Lassaletta et al., 2014). These losses are not only harmful to the environment, they pollute water and air, but also represent a major inefficiency in food production systems. Alarmingly, food waste exacerbates this problem, with projections suggesting that N losses from food waste could be six times higher by 2050 than in 1961 (Sutton et al., 2021).

In EU, agriculture continues to rely heavily on mineral fertilizers, with around 20 to 25 million tons used annually, while organic fertilizers account for only 5–10% of the market (Tur-Cardona et al., 2018). In Croatia, for example, around 88 000 tons of N fertilizer are used annually, but up to a third of this is lost to the environment (Špicnagel

Ćurko et al., 2021). There is clearly room and a need for a shift towards more efficient and sustainable nutrient management strategies.

One promising solution lies in bio-based fertilizers (BBFs), which are derived from recycled organic materials such as animal manure, food waste, compost, digestate and other agricultural by-products (Hansen, 2018; Troster et al., 2023; Kurniawati et al., 2023). These fertilizers not only recycle nutrients that would otherwise be lost, but also help to close the N cycle, a concept that is particularly important in modern agricultural systems where the decoupling of livestock and crop production has led to unbalanced nutrient flows (Klop et al., 2012; Vaneeckhaute et al., 2013, 2014; Sigurnjak et al., 2017). By returning N from animal manure to crop production, BBFs support the goals of a circular economy and reduce dependence on imported mineral fertilizers.

In contrast to mineral fertilizers, which only supply N in mineral form, BBFs often contain both mineral and organic N. The organic component can be taken up directly by the plants or made available through microbial processes such as N mineralization and immobilization (NMIT) (Inselsbacher et al., 2010). In addition, BBFs usually supply organic carbon to the soil, which stimulates microbial activity and promotes the NMIT process (Fontaine et al., 2003). The effectiveness of BBFs depends on several factors, including the carbon to nitrogen (C/N) ratio, the quality of the organic matter, the type of crop and the composition of the soil microbiome (Bardgett et al., 2003; Cheng, 2009; Alberquerque et al., 2012; Ninh et al., 2015; Bonanomi et al., 2019; Grunert et al., 2019).

The integration of BBFs into agricultural systems not only improves soil health and fertility, but also contributes to climate goals. Agriculture is one of the main sources of greenhouse gas emissions (Tubiello et al., 2013), but also holds enormous potential to become a more climate-friendly sector. For example, intensive livestock farms can set up biogas systems that convert manure into renewable energy and nutrient-rich digestate that can be reused as fertilizer. When properly managed, such systems offer economic, environmental and social benefits — from generating heat and electricity to promoting rural development and reducing pollution (Saedi et al., 2008).

Ultimately, the adoption of BBFs and circular agricultural practices offers a viable pathway to sustainable, resilient and efficient food systems. However, widespread adoption requires not only technological solutions, but also a cultural shift raising awareness among farmers, policy makers and consumers of the benefits of BBFs and resource-efficient practices (Shahpasand, 2014; Gontard et al., 2018; IFA, 2023; EEA, 2025). As we move towards 2050 and a population approaching about 10 billion people (Farooq et al., 2023), a rethink of how we manage nutrients and waste in agriculture will be essential not only to feed the world, but also to preserve the planet for future generations.

2.2. NITROGEN CYCLE IN AGROECOSYSTEM

The N cycle is an important part of how our planet functions, helping to support life in ecosystems on land and in the sea. It begins with a natural process called biological N fixation, in which certain microbes convert N from the air into forms that plants and animals can actually use (Galloway et al. 2004). These reactive N compounds, such as ammonia and nitrous oxide, move through the soil, water and living organisms before eventually being released back into the atmosphere through microbial processes (Galloway et al. 2003). However, in recent decades, human activities, particularly agriculture, industry and the burning of fossil fuels for the production of mineral fertilizers, have massively altered this natural cycle (Wayne, 1991; Giacomo, 2024). With the invention of the Haber–Bosch process, which enables the production of mineral fertilizers by fixing N from the air, the amount of reactive N in the environment has more than doubled compared to what nature used to provide itself (Isaksen et al., 2009). This has helped us to grow enough food for billions of people, but it has also caused problems (Erisman et al., 2008). Excessiev N can pollute rivers and lakes, damage ecosystems and contribute to climate change by favoring some species over others (Smil, 2004; Kuriyama & Hayashi, 2025). Nitrogen gasses such as NOx degrade air quality and can harm human health (Sprent, 1987), while nitrous oxide is a potent greenhouse gas and damages the ozone layer (Wayne, 1991). Nitrogen has helped feed the world, but at a high environmental cost (Erisman et al., 2013). Therefore, scientists are working to better understand how N moves around our world and how we can manage it more sustainably (Stevenson et al., 2006; Monks et al., 2009).

The amount of N in the soil is not fixed, but depends on natural factors such as climate, vegetation, topography, the type of parent rock from which the soil was formed and the age of the soil. Nitrogen is present in both organic and inorganic forms, with organic nitrogen accounting for the largest proportion, between 50% and 99% of the total. This organic N is mainly stored in humus and in the remains of plants and animals that are still decomposing. In order for plants to actually use this N, it must undergo a biological process called mineralization, which is slow, typically 1–3 % per year (Bergmann, 1992; Rengel et al., 2023; Herak Ćustić et al., 2025). Since only a small proportion of the N in the soil is readily available in mineral form, farmers often have to apply it through fertilizers to meet the nutrient requirements of the plants.

Plants take up N mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-) ions, which together account for around 70 % of all nutrients taken up by their roots. Whether there is more ammonium or nitrate in the soil depends on conditions such as oxygen content, temperature, soil moisture and pH value. Well-aerated soils tend to contain more nitrate, while ammonium tends to be found in more humid, oxygen-poor environments. Dry weather can cause nitrate to accumulate near the surface, while heavy rainfall, especially on sandy soils, can lead to nitrate leaching, which is not only a loss for the plant but also a problem for the environment. Urea, a common fertilizer, also provides N, but is generally broken down by soil microbes before plants can absorb it. As nitrate moves easily with water, it is quickly washed out, while ammonium adheres to soil particles due to its positive charge and remains more stable (Bergmann, 1992; Rengel et al., 2023; Herak Ćustić et al., 2025).

Once N enters the soil, it becomes part of a complex and dynamic cycle, a local part of the much larger global N cycle. This N cycle (Figure 1) in soil includes key processes such as mineralization, immobilization, nitrification, denitrification, ammonia volatilization, plant uptake, leaching, erosion and surface runoff (Salomez, 2004;

Butterbach-Bahl et al., 2011). During mineralization, soil microbes convert organic N into ammonia (NH_3), which turns into ammonium (NH_4^+) in most soils, except in highly alkaline soils. The rate of this process is highly dependent on temperature, moisture and the amount of oxygen available, cool or dry conditions slow it down (Salomez, 2004). On the other hand, immobilization occurs when microbes take up nitrate and ammonium for their own growth, making them temporarily unavailable to plants, especially when decomposing N-poor material. After mineralization, the ammonium can be converted to nitrate by nitrification, a microbial process in which nitrate is formed via nitrite (NO_2). As nitrate is highly mobile in water, this step significantly increases the risk of N loss from the soil. When soils become depleted of oxygen, denitrification can occur, in which nitrate is converted into gasses such as nitric oxide (NO), nitrous oxide (N_2O) and nitrogen gas (N_2), which escape into the atmosphere. Another way in which N can be lost is through the volatilization of ammonia, which is more common in high pH soils and in hot or windy conditions. Leaching is particularly problematic because, unlike many other nutrients, nitrate does not bind well to soil particles and can easily be washed out by rainfall or irrigation (Butterbach-Bahl et al., 2011; Herak Ćustić et al., 2025). In addition, soil erosion can entrain N-rich particles, especially organic nitrogen and ammonium, which are bound to fine clay in sloping or hilly areas. Surface runoff can also remove dissolved N, although this is usually less significant than leaching (Salomez, 2004).

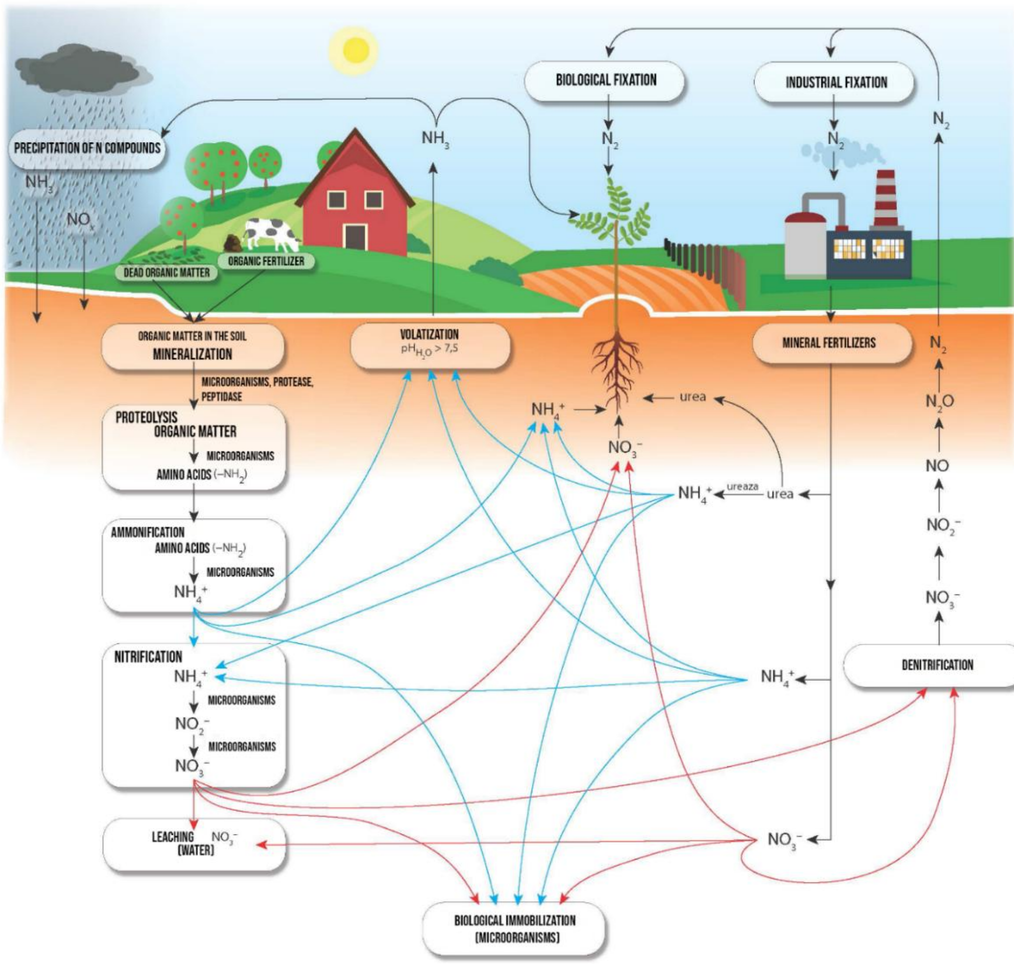

Figure 1. Nitrogen cycle in the system-soil-plant-atmosphere (source: Herak Ćustić et al., 2025)

Different plants have different preferences when it comes to N. While most crops prefer nitrate, others such as potatoes, grasses and some forest species thrive on ammonium. In general, plant growth is best when both forms are present in balance. The form of N absorbed also affects the soil near the roots (the rhizosphere): When plants take up ammonium, they release hydrogen ions that acidify the surrounding soil; when they take up nitrate, the soil tends to become more alkaline. As the natural N cycle is often disturbed in agriculture — especially through the use of fertilizers, wise management of ammonium and nitrate is key to effective plant nutrition and the protection of soil and water quality (Salomez, 2004; Butterbach-Bahl et al., 2011; Herak Ćustić et al., 2025).

2.3. ANAEROBIC DIGESTION

Biogas and digestate are produced in biogas plants by anaerobic digestion (AD). While biogas can substitute fossil fuels, digestate can be assessed as bio-based fertilizer and potentially substitute for mineral fertilizers (Giacomo, 2024).

AD is a complex biological process that takes place under oxygen-free conditions and involves a series of biochemical steps that convert organic matter into biogas. The process consists of four main steps/stages (Figure 2): Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis (Dussadee et al., 2016; Sigurnjak et al., 2017; Przygocka-Cyna & Grzebisz, 2020; Jankauskienė et al., 2024). Each stage is carried out by different groups of microorganisms, each with different physiological and nutritional needs, making it a delicate system that requires a balance to function effectively (Stark & Richards, 2009; Sigurnjak et al., 2017; Sutton et al., 2022).

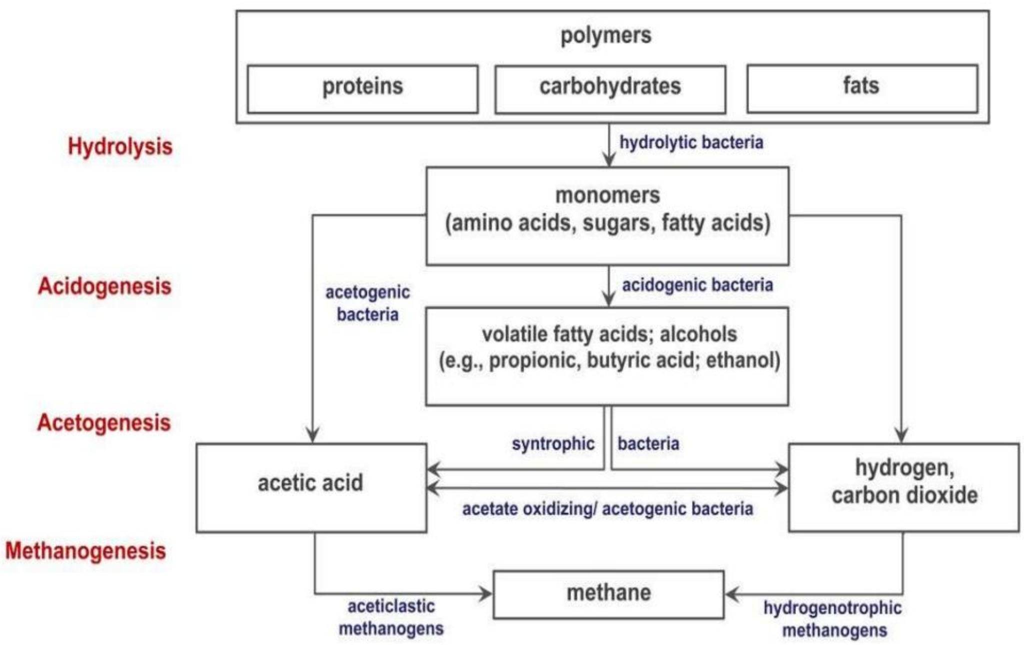

Figure 2. Flow diagram of the anaerobic digestion process (source: Technische Universität Dresden, 2025)

Hydrolysis is the first step in which large, insoluble organic molecules such as carbohydrates, fats and proteins are broken down into simpler, soluble compounds such as sugars and amino acids. This step is crucial as microorganisms cannot directly utilize complex molecules. Hydrolytic bacteria — including Clostridium, Bacteroides and Eubacterium, form extracellular enzymes that break down complex polymers into absorbable units (Schnurer & Jarvis, 2009; Stark & Richards, 2009; Sutton et al., 2022). The efficiency of this step depends on the type of substrate; proteins, for example, are broken down faster than cellulose (Stark & Richards, 2009; Bhatia, 2014; Sutton et al., 2022).

This is followed by acidogenesis, in which the hydrolysis products are fermented by acidogenic bacteria to alcohols, volatile fatty acids (VFAs), acetate, carbon dioxide (CO_2) and hydrogen (H_2). This phase is rapid, with the bacteria usually regenerating in less than 36 hours, but it can lead to an accumulation of volatile fatty acids if it is not properly balanced, which can lead to a drop in pH and instability of the process (Stark & Richards, 2009; Bhatia, 2014; Sutton et al., 2022).

In the acetogenesis phase, VFAs and alcohols that cannot be directly utilized by methanogens are converted by acetogenic bacteria into acetic acid, H_2 and CO_2. These reactions are thermodynamically feasible only at low H_2 partial pressure and require close cooperation with methanogens that consume H_2 in the next step — a symbiotic relationship known as interspecies H_2 transfer (Schink, 1997; Deublein & Steinhauser, 2008; Schnurer & Jarvis, 2009; Chandra et al., 2012; Bhatia, 2014).

Finally, methanogenesis is the final stage in which the methanogenic archaea, a group of single-celled microorganisms, convert acetate, CO_2 and H_2 into methane. This step is both critical and the slowest in the AD process, and its performance often determines the overall efficiency and stability of the system (Seadi et al., 2008; Stark & Richards, 2009; Bhatia, 2014; Sutton et al., 2022).

Maintaining the balance between acid-forming and methane-forming microbes is key to successful digestion. An overload in one phase can suppress the other, leading to instability and lower methane yield. To solve this problem, process engineers often use phase separation techniques such as membrane filtration, kinetic control or pH regulation (Pohland & Mancy, 1969; Ghosh & Pohland, 1974; Massay & Pohland, 1978; Cohen et al., 1979; Fernandez, 1986). Some systems even physically separate microbial groups to optimize their growth and function (Pohland & Ghosh, 1971).

In addition to energy production, AD also help reduce greenhouse gas emissions, particularly N_2O by capturing the biogas in a closed system. The remaining digestate is a valuable organic fertilizer that retains most of the N and other nutrients from the original manure or biomass (Stark & Richards, 2009; Sigurnjak et al., 2017; Sutton et al., 2022).

Although AD is often emphasized for its ability to produce renewable energy, more attention is being paid to its by-product digestate which is rich in nutrients and has great potential to be substitute for mineral fertilizers. Therefore, it is important to understand its composition, benefits for soil and plants (advantages and disadvantages) and its safe use.

2.4. DIGESTATE

Digestate, the nutrient-rich by-product of AD, is increasingly recognized as a valuable substitute for mineral and animal fertilizers, especially in the context of the growing framework of sustainable and circular agriculture (Makadi et al., 2012; Sigurnjak et al., 2017; Chojnacka et al. 2020).

In recent years, digestate has gained increasing attention as a promising bio-based material that can partially or completely replace mineral fertilizers. In order for digestate to be used as a fertilizer, both its quality and the quality of the feedstock entering the AD process must be carefully controlled. When used as a fertilizer in the field, it is important to determine the chemical composition, agronomic properties, overall quality and efficiency, as well as to assess the potential health and safety risks through crop trials (Haraldsen et al., 2011). Both field and laboratory studies have been conducted to evaluate the agronomic performance and environmental impact. Several of these studies have shown that digestate can meet or exceed the effectiveness of mineral N fertilizers in terms of crop yield and nutrient availability (Walsh et al, 2012;

Vaneeckhaute et al., 2014; Cavalli et al., 2014; Cavalli et al., 2016; Riva et al., 2016; Sigurnjak et al., 2017; Panuccio et al., 2018). In addition it improves soil quality and increases crop yields between 15 and 28% (Moller & Muller, 2012; Lopedota et al., 2013; Monlau et al., 2016).

It is derived from the breakdown of various organic materials (feedstock) such as animal manure (pig, cattle, poultry, etc.), crop residues, sewage sludge and food waste (Alburquerque et al., 2012; Reuland et al., 2022), and its physical form, liquid or solid fraction, depends largely on its dry matter content (Møller et al., 2015). What makes digestate agronomically valuable is its high concentration of plant-available nutrients, especially mineral form of N-ammonium (NH_4^+), along with essential macro- and micronutrients (EC, 2014b; Tambone et al., 2017; Hammerschmiedt et al., 2022; Kovačević et al., 2022).

Compared to raw manure, digestate contains more N in a form that plants can easily absorb, which improves short-term nutrient uptake. However, this also leads to a higher risk of ammonia emissions (NH_3), as the AD process tends to increase the pH of the material (Webb et al., 2013). These emissions can be effectively reduced through proper application techniques such as soil injection or incorporation (Riva et al., 2016), which also improve N use efficiency (NUE). In some cases, studies have shown that the NUE of digestate can match that of mineral fertilizers, although the long-term effects on soil and crop performance are not yet fully understood (Tampio et al., 2016).

Eventhough, its potential as a sustainable fertilizer, the use of digestate on agricultural land still increases some agronomic and environmental concerns (Piccoli et al., 2023). One of important challenges lies in the variability of its quality and characteristics such as pH, organic matter content and nutrient composition can significantly affect its effectiveness and safety. Plus, digestate from waste materials can contain environmental contaminants such as pathogens, heavy metals, pesticide residues, steroid hormones and other organic pollutants that can pose risks to soil health and the entire ecosystem (Goberna et al., 2011; Le Maréchal et al., 2019; Nag et al., 2019; Roccotelli et al., 2020; Golovko et al., 2022). According to Formowitz and Fritz (2010), applying digestate too early in the soil and before the plants can actively use the nutrients, can lead to nutrient losses through leaching into deeper soil layers or nitrate (NO_3^-) emissions into the groundwater. If the pH value of the digestate has a pH higher than 8, there is also an increased risk of volatilization losses, particularly of ammonia. Consequently, to ensure both the safety and efficacy of the use of digestate, it is essential to control the quality and origin of the feedstock (Moller & Muller, 2012) and apply appropriate post-treatment procedures. Equally important is a thorough analysis of the chemical composition before application to the soil, as improper use could lead to contamination of the soil and pose a risk to the food chain (Monlau et al., 2015).

Digestate is typically separated into two main fractions (Figure 3) with uneven nutrient distribution, each with its own agricultural benefits. The liquid fraction of digestate (LDF) is rich in N (65-70%) and K (70-80%) and is therefore well suited for direct fertilization, while the "fibrous "solid fraction of digestate (SFD) has a higher content of P (55-65%) and organic carbon (60-70%) and is suitable as a soil amendment (Goberna et al., 2011; Al Seadi et al., 2012; Fuchs & Drosg, 2013; Sigurnjak et al., 2017). Since LFD has more N in mineral form – ammonia, it is suitable as a potential N fertilizer.

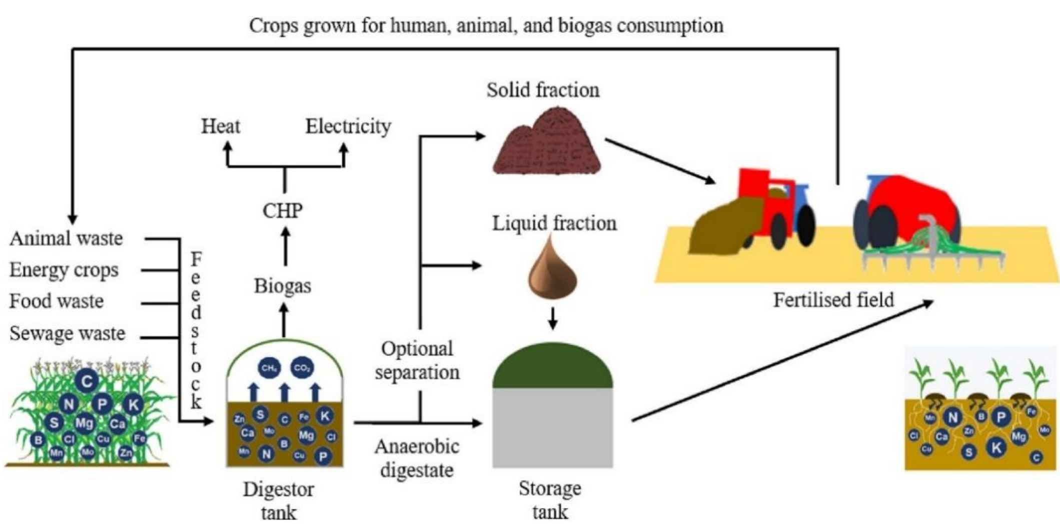

Figure 3. Utilization of Anaerobic Digestate as Fertiliser: Process and End-Use (source: Van Midden et al., 2023)

The remaining organic material can also help to improve soil structure and support microbial activity (Van Midden et al., 2023).

As there are two types of AD, thermophilic digestion has been shown to effectively reduce pathogens and meet EU hygiene standards, while digestate from mesophilic processes may require additional treatment to ensure safety (Paavola & Rintala, 2008; Sahlström, 2003).

Moreover, advanced analytical techniques such as nuclear magnetic resonance and Fourier transform infrared spectroscopy have shown that while AD stabilizes organic matter, it also leads to the accumulation of more resistant compounds such as lignin and humic acids. These contribute to the potential of digestate for long-term improvement of soil health (Schievano et al., 2009; Gómez et al., 2011). However, variations in digestate quality, both between different biogas plants and within individual batches, remains a major limitation (Lukehurst et al., 2010). This highlights the urgent need for clear quality standards, improved processing methods and well-planned utilisation strategies for digestate. Further research, supportive policy development and increased awareness among farmers and other key stakeholders are needed to realise the full potential of digestate as a reliable and sustainable source and substitute of N in agriculture (Lamolinara et al., 2023).

2.5. NITROGEN AVAILABILITY

Understanding the availability of nitrogen (N) from organic sources such as animal manure and digestate is critical for improving nitrogen use efficiency (NUE) in crops while minimizing environmental losses (Cavalli et al., 2016). To assess this efficiency, researchers commonly use two key indicators: apparent nitrogen recovery (ANR) and nitrogen fertilizer replacement value (NFRV). The ANR measures the proportion of applied nitrogen taken up by plants beyond what is absorbed in unfertilized controls, while the NFRV, also referred to as mineral fertilizer equivalence compares the effectiveness of organic and mineral N sources by expressing the ratio of their respective ANR (Schröder, 2005). Numerous field trials and laboratory incubations have supported the use of these indicators in the evaluation of N uptake from untreated and digested manures (Van Kessel & Reeves, 2002; Sørensen et al., 2003; Schröder et al., 2005, 2007, 2013; Morvan et al., 2006; Reijs et al., 2007; Chantigny et al., 2008; de Boer, 2008; Möller et al., 2008; Bechini & Marino, 2009; Saunders et al., 2012; Herrmann et al., 2013; Sieling et al., 2013). These studies consistently find that N availability in the first year from manure and digestate use is largely correspondent to their ammonium content (NH_4^+-N), which means that the NFRV is very close to the ratio of NH_4^+-N to total N (Möller & Müller, 2012; Webb et al., 2013). In addition, repeated manure applications over time contribute to increased N availability in later years thanks to the gradual mineralization of organic N and the remineralization of immobilized NH_4^+-N (Gutser et al., 2005; Nevens & Reheul, 2005; Hernández et al., 2013).

Although ^{15}N isotope labeling is the most accurate method for tracking nitrogen in soil-plant systems, it is often impractical due to its high cost (Vellinga & Andre, 1999). Instead, simpler methods based on ANR calculations from fertilized and unfertilized treatments are often used to estimate NUE and NFRV. Field trials are ideal for this purpose, but their labor-intensive and costly nature often leads researchers to rely on controlled laboratory incubations. These experiments provide a practical way to monitor N release over short periods of time and serve as a reliable tool for an initial estimate (De Neve, 2017; Rigby et al., 2016). However, such methods do not take into account the influence of vegetation on N dynamics. Biological factors such as plant uptake, rhizodeposition, microbial turnover in the rhizosphere and root-induced changes in soil physical properties can significantly influence N availability and movement (Czarnes et al., 2000; Inselsbacher et al., 2010; Cesco et al., 2012; Palacios et al., 2014; Coskun et al., 2017; Meier et al., 2017). Despite their importance, direct comparisons between planted and unplanted systems with bio-based fertilization are still rare, highlighting the need for more integrative research in this area.

2.6. AGRONOMIC, ENVIRONMENTAL AND ECONOMIC BENEFITS

In recent years, the use of digestate has become increasingly important due to its potential agronomic, environmental and economic benefits. From an agronomic point of view, digestates are a valuable organic fertilizer due to their high content of plant-available nutrients, mainly N in the ammonium form (mineral) as well as P and K, as already mentioned. Studies have shown that their application can significantly increase crop productivity, with reported yield increases ranging from 15 % to 50 %, depending on the type of crop, soil conditions and the composition of the digestate, i.e. the feedstock going into the digestion (Möller & Müller, 2012; Lopedota et al., 2013; Monlau et al., 2016). Digestate contributes to improved soil structure, increased microbial activity and organic matter enrichment, all of which have a positive effect on long-term soil health (Müller-Stöver et al., 2016; Sigurnjak et al., 2017). Incorporating digestate into the soil can support carbon sequestration by enhancing the soil's ability to capture and store carbon dioxide from the atmosphere, thereby contributing to climate change mitigation (ADC, 2025).

From an environmental perspective, the use of digestate reduces dependence on mineral fertilizers, which are often energy-intensive to produce and contribute to greenhouse gas emissions. The production and application of digestate helps close nutrient loops by recycling organic waste, thereby supporting the circular economy (Figure 4) and reducing nutrient losses to air and water (Stoknes et al., 2016; Krzyżaniak et al., 2018), which is crucial for today's sustainability. When properly managed, digestate can also mitigate environmental problems such as nitrate leaching and ammonia volatilization, although its use requires careful planning to avoid such risks (Haraldsen et al., 2011; Golovko et al., 2022).

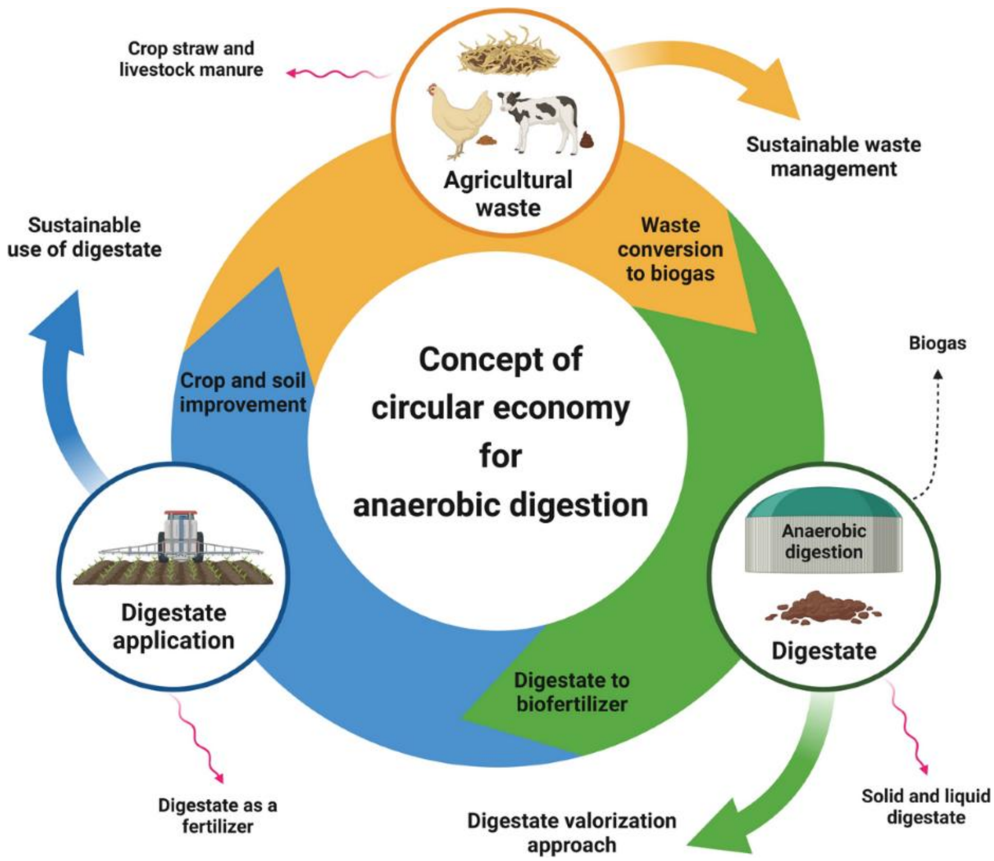

Figure 4. Integrating Digestate into Sustainable Farming Practices (source: Spring Nature, 2025)

From an economic perspective, there is little information in the literature to determine the economic value of digestate, but digestate offers a cost-effective alternative to mineral fertilizers (Lamolinara et al., 2023), especially in regions with well-developed biogas infrastructure (such as Germany and the Netherlands). Its use not only reduces input costs for farmers, but also increases the value of agricultural waste streams, making it a practical component of sustainable and integrated nutrient management systems (Rigby & Smith, 2013; Coelho et al., 2020).

2.7. STATE OF ART IN CROATIA WITH FERTILIZER REGULATIONS

Today, Croatia has an installed biogas capacity of around 48 MW, and the electricity generated is sufficient to cover the needs of around 100 000 households. On average, a single biogas plant with a capacity of 1 MW generates around 8 000 MWh of electricity per year, which means that the entire biogas sector in Croatia supplies around 384 000 MWh per year. This represents a significant contribution to the national energy balance and saves millions of euros that would otherwise be spent on electricity imports. Despite this valuable contribution, the Croatian biogas sector is facing increasing challenges, especially since the end of 2021. After the outbreak of the war in Ukraine and the resulting global energy crisis, the operating costs for biogas plants, especially for raw materials have doubled, putting producers under immense financial pressure. Many biogas operators who were unable to cover these rising costs were forced to reduce back production just to keep their plants in operation (HORTE, 2022).

Throughout 2022, the association of Renewable Energy Sources (RES) of Croatia repeatedly drew the attention of the national authorities to the growing crisis and even sent urgent letters to the Prime Minister of the Republic of Croatia. In an attempt to defuse the situation, the RES drafted a proposal in July 2022 to adjust the subsidy rates to the increased cost of raw materials. Although the proposal was submitted to the Ministry of Economy and Sustainable Development, it is still pending. During this period, many biogas plants with fixed electricity sales contracts via Croatian Energy Market Operator (HORTE) were at a particular disadvantage. While electricity prices on the European stock markets soared, Croatian biogas plants continued to receive a fixed subsidy rate of EUR 170 per MWh, which was far below the amount needed to cover operating costs. As a result, some operators left the incentive scheme to sell electricity on the free market, where they could negotiate higher prices and ensure the financial viability of their operations. At the end of 2022, only 23 biogas plants with a capacity of around 22 MW i.e. around half of the national biogas sector were still covered by the HROTE support framework (HROTE, 2019). This situation makes it clear that regulatory adjustments and targeted financing mechanisms are urgently needed to ensure the survival and further development of the Croatian biogas sector. The use of fertilizers is strictly regulated in the European Union to ensure environmental protection, sustainable agriculture and long-term food security. The EU Fertilizer Regulation (2019/1009), which sets harmonized standards for the production, quality, labeling and safety of organic and BBFs, strongly supports the promotion of

BBFs, including digestate. This regulation plays a crucial role in promoting nutrient recycling, thereby reducing dependence on mineral inputs and promoting the circular economy. In parallel, the Nitrates Directive (91/676/EEC) aims to limit N pollution by limiting the application of livestock manure with mineral fertilizers to 170 kg N ha^{-1} per year and promoting precision farming techniques.

At the same time, the use of fertilizers in Croatia, which is closely linked to the use of digestate, is regulated by a comprehensive European and national legal framework designed to ensure environmental protection, sustainable agriculture and food security. At EU level, the Fertilizer Regulation (2019/1009) sets harmonized standards for the production, quality, labeling and safety of organic and bio-based fertilizers (BBFs), which strongly promote nutrient recycling and support the principles of the circular economy. In addition, the Nitrates Directive (91/676/EEC) aims to reduce nitrate pollution from agriculture by limiting N application from organic and mineral fertilizers to 170 kg N ha^{-1} per year and promoting the introduction of precision farming techniques. These initiatives are reinforced by the EU Circular Economy Action Plan, the European Green Deal and the Farm-to-Fork Strategy, which together aim to reduce nutrient losses by 50% and the use of mineral fertilizers by 20% by 2030. In this context, the RENURE (REcovered Nitrogen from manURE) criteria present an important regulatory opportunity to improve nutrient management while ensuring environmental sustainability. RENURE refers to processed manure-derived products (digestate, liquid fraction of digestate, etc.) that meet defined chemical and agronomic thresholds and can therefore be applied under conditions similar to those for mineral fertilizers within the framework of the Nitrates Directive. By allowing the use of validated RENURE materials beyond the conventional limit of 170 kg N ha^{-1} in nitrate vulnerable zones, this approach contributes to higher N use efficiency, reduces reliance on mineral fertilizers, and strengthens nutrient recycling. In Croatia, where biogas production generates considerable amounts of digestate and agriculture faces high fertilizer costs, implementing RENURE criteria could enhance the agronomic value of digestate and contribute to both the economic viability of the biogas sector and the sustainability of agricultural production.

At national level, Croatia has adapted its legislation accordingly and introduced measures such as the Fertilizer Act (NN 39/2023), the Ordinance on the Use of Sewage Sludge in Agriculture (NN 38/2008), the Ordinance on the Protection of Agricultural Land from Pollution (NN 71/2019) and the Ordinance on the Action Program for the Protection of Waters from Nitrates from Agricultural Sources (NN 72/2021). The amendment to the Agricultural Land Act (NN 52/2022) also strengthens national efforts to promote sustainable land management and the responsible use of fertilizers (Šatvar Vrbančić et al., 2025).

Nevertheless, Croatia continues to face major challenges in maintaining soil health. Soil degradation (Biernat et al., 2012; Comparetti er al., 2013) and erosion threaten around one million hectares of agricultural land, significantly more than the EU average. Recent data from the Croatian Agency for Agriculture and Food shows that 90% of soil samples analyzed contained less than 3% humus and 55.4% even had less than 2% humus (Croatian Agency for Agriculture and Food, 2023). As well in Croatia, over 50 % of all agricultural land consists of acidic soils, with the proportion in the Pannonian region being over 90% (Bogunović et al., 1996; Lazarević et al., 2014). The decline in soil pH is not just a chemical problem, but a direct result of many years of intensive agricultural practices. Years of excessive use of mineral fertilizers, topsoil erosion and lack of organic matter restoration have gradually acidified the soil. As a result, crop production today faces major challenges: lower yields and availability and solubility of nutrients make it difficult for plants to thrive (Bogunović & Kisić, 2017). Addressing this crisis requires a shift toward more sustainable agriculture, in which BBFs such as digestate could play a key role in restoring soil fertility, improving resilience to climate change and ensuring the long-term sustainability of Croatian agricultural production.

3. MATERIALS AND METHODS

3.1. FIELD EXPERIMENT

3.1.1. Field experiment setup

Field experiments were conducted for two consecutive years, 2018 and 2019 at the University of Zagreb Faculty of Agriculture on silt−loam soil. Figure 5 shows a fragment of the map with the exact location of the experimental field Maksimir (45°49'39, N; 16°02'02, E), at an altitude of 127 m.

Figure 5. Location and section of the experimental field (source: L. J. Telak, 2019; Google Earth, 2024.)

3.1.2. Experimental design and fertilization treatments

The experiment was established in plots of 39.2 m^2 per replicate arranged in a quadruplicate randomized block design, involving 7 treatments:

1 – unfertilized control (C);

2 – synthetic mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN);

3 – liquid cattle manure (LCM);

4 – solid fraction of digestate (SFD);

5 – liquid fraction of digestate (LFD);

6 – a mix of SFD+NPK;

7 – a mix of LFD+NPK.

Treatments were spread across the experimental field to minimize potential influence of variable soil conditions on the results.

As a mineral reference fertilizer, nitrogen-phosphorus-potassium (NPK – ammonium nitrate based fertilizer) and calcium-ammonium-nitrate, which are the most commonly used mineral fertilizers in Croatia, were applied in combination with both digestate fractions, SFD and LFD.

The application dosage was set at 140 kg of total N in all fertilization treatments (suboptimal concentration presented in Table 1.). Before fertilization ≈30 kg of mineral N was determined in the soil. Also, maize was sown after maize and there was no catch crop in between. Since experimental field falls under the Nitrate Voulnerable Zones (NVZ) (Official Gazette NN 60/2017; Ondrašek et al., 2021) which are presented in the Figure 6, dosage of 140 kg N ha^{-1} was applied in order to prevent subsequent nitrate leaching after harvest, even though Nitrates Directive allows 170 kg total N ha^{-1}.

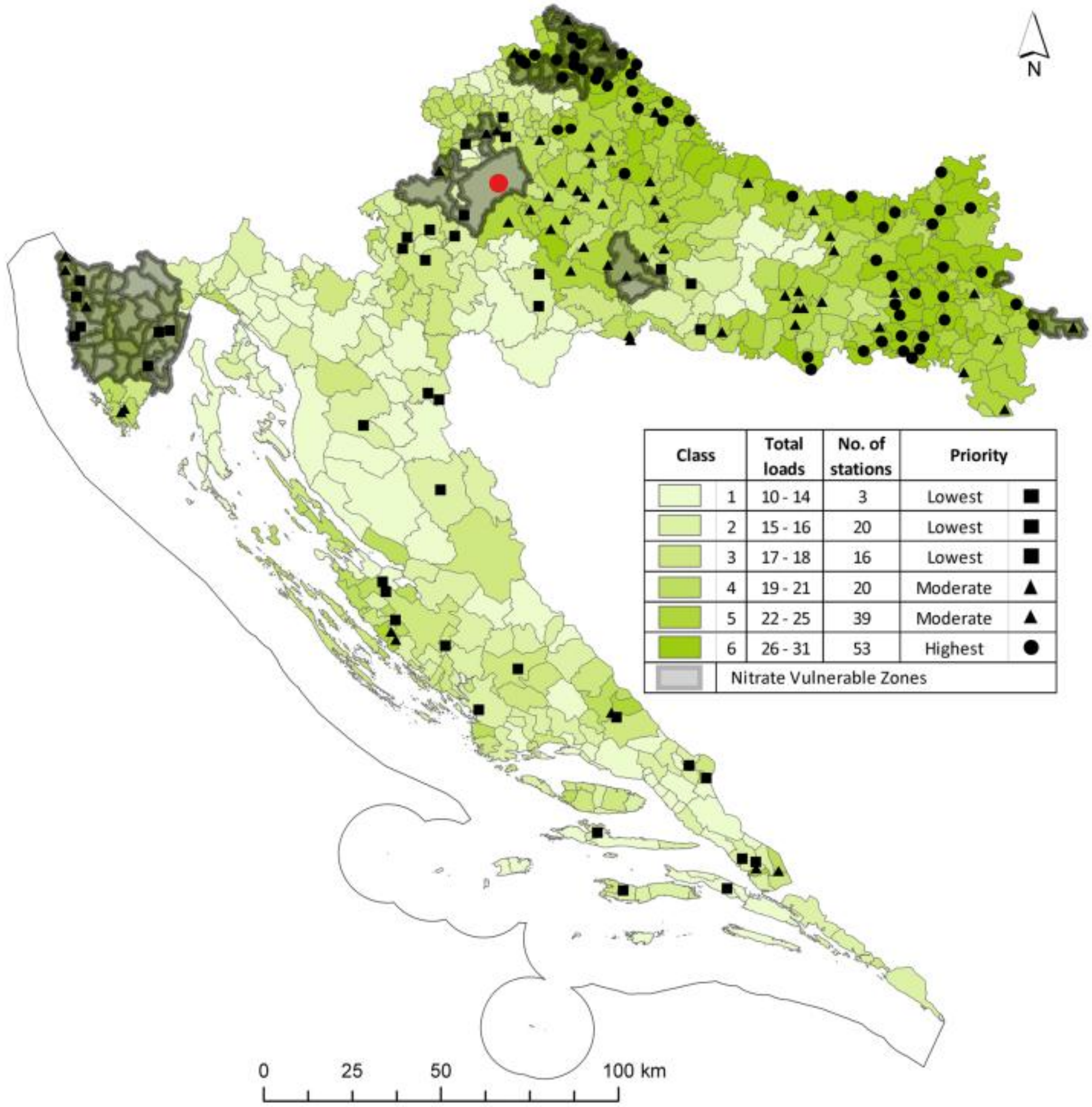

Figure 6. Priority Map of 151 New Groundwater Monitoring Stations for the Nitrates Directive in Croatia Based on Agro-Environmental Pollution, red dot is experimental field (source: Ondrašek et al., 2021)

Nutrient application rates for the different treatments over two consecutive years are summarized in Table 1.

Table 1. Dosage of total nitrogen (kg ha^{-1}) applied for the seven different fertilization treatments; P_2O_5 and K_2O amount were brought to the field via application of original fertilization regime of total nitrogen; additional application of synthetic CAN (kg ha^{-1}) in order to satisfy synthetic mineral fertilization

Treatment	Year	kg ha^{-1}											
		available N in the soil	added N to the soil						available and added N	NH_4-N	NO_3-N	P_2O_5	K_2O
		BF	NPK	CAN	LCM	SFD	LFD	sum of N	sum of N				
C	2018	30	-	-	-	-	-	0	30				
	2019	30	-	-	-	-	-	0	30				
NPK + CAN[a]	2018	30	70	70	-	-	-	140	170	79	61	70	70
	2019	30	70	70	-	-	-	140	170	75	65	70	70
LCM	2018	30	-	-	140	-	-	140	170	20	-	120	68
	2019	30	-	-	140	-	-	140	170	47	-	100	24
SFD	2018	30	-	-	-	140	-	140	170	7	-	31	36
	2019	30	-	-	-	140	-	140	170	26	-	53	44
LFD	2018	30	-	-	-	-	140	140	170	14	-	60	21
	2019	30	-	-	-	-	140	140	170	60	-	117	24
SFD+NPK[a]	2018	30	70	-	-	70	-	140	170	47	26	85	88
	2019	30	70	-	-	70	-	140	170	53	30	97	92
LFD+NPK[a]	2018	30	70	-	-	-	70	140	170	51	26	100	80
	2019	30	70	-	-	-	70	140	170	69	30	129	82

[a] 50% of total N (140 kg N ha^{-1}) from NPK and 50% of N from CAN was added to the soil; 50% of total N (140 kg N ha^{-1}) from SFD and 50% of N from NPK was added to the soil; 50% of total N (140 kg N ha^{-1}) from LFD and 50% of N from NPK was added to the soil; C: unfertilized treatment; NPK: ammonium nitrate based fertilizer; CAN: calcium-ammonium-nitrate based fertilizer; LCM: liquid cattle manure; SFD: solid fraction of digestate; LFD: liquid fraction of digestate.

Each year all fertilizers were applied on the same day, 27 April 2018 and 2 May 2019. LFD, and LCM were delivered to experimental site by truck in big 1000 L plastic container. Before application they were mixed so that fertilizer mixture was as much as homogeneous. LCM, SFD and LFD were added to soil manually in order to assure accurate dosage. Mineral fertilizers (NPK+CAN) were also delivered to experimental site in bags that were previously weighed in the laboratory. After application, the fertilizers were immediately incorporated into soil by rotary harrow to reduce the ammonia volatilization and 2 days later, on 29 April, sowing took place, while in 2019, sowing took place next day, 3 May. As a test crop, fodder maize (*Zea mays* L.) hybrid R 0725 (Corteva agriscience, FAO vegetation group 570) was used. Desired plant populations 80 000 plant ha^{-1} were achieved by overplanting and thinning at the V3 to V4 growth stages (Abendroth et al., 2011). In each plot, maize was sown in 8 rows, 0.7 m inner row space and 6 m long. Only 4 inner rows were harvested and analyzed. Maize was harvested on 28 September 2018 and 1 October 2019 (end of the experiment for each year). During vegetation period all management practices have been implemented, by that cultivation, weed and pest control.

3.1.3. Climate conditions

The review of climatic conditions for the research period was carried out on the basis of data collected at the nearest meteorological station (Figure 7) Zagreb - Maksimir (45°49'14,N 16°02'15,E). Weather conditions for temperature and precipitations were taken from Croatian Meteorological and Hydrological Service (DHMZ) during year 2018 and 2019.

Figure 7. Meteorological station Zagreb – Maksimir (source: Google Earth, 2024.)

Croatia's climate is determined by its location in the northern temperate latitudes and associated by large and medium scale weather processes. Continental climate of Croatia has a moderately continental climate and is located all year round in the circulation belt of moderate latitudes, where the state of the atmosphere is very variable: it is characterized by a variety of weather situations with frequent and intense changes throughout the year (DHMZ, 2024.).

Climate classifications are most often carried out according to the Köppen and Thornthwaite classifications.

3.1.4. Precipitations

In addition to other meteorological elements, precipitation has a dominant influence on plant production. The choice of tillage system and appropriate plant production systems can partially compensate the lack of precipitation in areas where it occurs. The results of plant production largely depend on the amount, distribution, frequency and intensity of precipitation.

The analysis was carried out for the months of April, May, June, July, August, September and October, which represents the period from maize sowing to maize harvest. The annual course of the amount of precipitation (mm) during maize vegetation growth through 70th year period was 569.7 mm (Pucarić et al., 1997) while during 2018 was 535.8 mm and in 2019 was 625.5 mm (DHMZ, 2019).

The average course of the amount of precipitation (mm) during maize vegetation period (Table 2), for 2018 from April to October was 76.5 mm and for 2019 was 89.4 mm (DHMZ 2018; 2019). According to Pucarić et al. (1997) average precipitation during maize growth from April to October is 81.4 mm. The highest amount of precipitation in 2018 was recorded in June (127.8 mm), while the lowest in August (40.7 mm). In 2019 highest amount of precipitation was recorded in September (150.1 mm) and lowest in October (42.3 mm).

Table 2. Annual and average (Pucarić et al., 1997) precipitation for Maksimir experimental field in 2018 and 2019 during the maize growing season (DHMZ, 2018; 2019.)

Year	Precipitation mm												Yearly	APMP*
	I	II	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	XI	XII		
70-yr average				61.5	78.0	97.2	80.8	87.0	89.3	75.9				569.7
2018	567	87.5	72.2	65.8	68.7	127.8	85.2	40.7	59.0	88.6	80.4	21.0	853.6	535.8
2019	35.8	25.1	36.8	81.1	147.7	70.8	76.8	56.7	150.1	42.3	179.2	98.1	1000.5	625.5

*APMP – the annual course of the amount of precipitation (mm) during maize vegetation period

According to percentile ranks and classification ratings, the precipitation amounts for 2018 and 2019 have been described in the following Figures the following categories: extremely dry, very dry, dry, normal, wet, very wet and extremely wet.

According to percentile ranks and classification ratings, the precipitation amounts for experimental field during 2018 in April have been described as normal, for May normal, for June dry, for July normal, for August dry, for September dry and for October normal.

According to percentile ranks and classification ratings, the precipitation amounts for experimental field during 2019 in April have been described as wet, for

May wet, for June normal, for July normal, for August normal, for September wet and for October dry.

3.1.5. Temperature

Temperature is an important climatic factor that significantly influences plant growth. Its annual fluctuations shape the vegetation cycle and, together with precipitation, play a decisive role in the structure and productivity of plant production systems in a particular region. Maize grows fastest on temperature around 30°C with enough amount of precipitations what is always not a case. Best temperature for maize growth is from 24 to 29°C. Lowest limit of temperature for germination is 12-13°C, and upper limit is 40-45°C according to Pucarić et al. (1997). According to Pucarić et al. (1997) the optimal average temperature during maize growth from May to September is 21.5°C.

The analysis for temperature was also carried out for the months of April, May, June, July, August, September and October, which represents the period from maize sowing to maize harvest. During vegetation period (from April to October), average air temperature in 2018 was 19.2°C and in 2019 was 18.1°C.

The highest temperature in 2018 was recorded in August (23.7°C), while the lowest in October (13,7°C). In the 2019 highest temperature was recorded in June (23.8°C) and lowest in April (12.4°C) are shown in Table 3.

Table 3. Average air temperature for Maksimir experimental field in 2018 and 2019 during the maize growing season (DHMZ, 2018; 2019.)

Year	Temperature °C												Annual value
	I	II	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	XI	XII	
70-yr average				11.3	15.9	19.4	21.1	20.4	16.2	11.0			
2018	5.2	0.2	5.2	16.1	19.5	21.4	22.5	23.7	17.7	13.7	7.9	2.8	13.0
2019	1.5	4.8	9.5	12.4	13.7	23.8	22.9	23.5	17.2	13.2	9.2	4.6	13.0

According to percentile ranks and classification ratings, thermal conditions in Croatia during 2018 and 2019 fall in the following categories: extremely warm, very warm, warm, normal, cold, very cold and extremely cold.

According to percentile ranks and classification ratings, thermal conditions in Croatia for experimental field during 2018 for April falls as extremly warm, for May extremly warm, for June extremly warm, for July very warm, for August extremly warm, for September very warm and for October extremly warm.

According to percentile ranks and classification ratings, thermal conditions in Croatia for experimental field during 2019 for April falls as normal, for May very cold, for June extremly warm, for July very warm, for August very warm, for September warm and for October very warm.

3.1.6. Soil water balance by Thornthwaite

The water balance in the soil was calculated using Thornthwaite's method, determining both potential and actual evapotranspiration, as well as identifying periods of water deficit and surplus. According to the average values for years 2018 and 2019 (Table 4) the actual evapotranspiration amounts to 644.3 mm in 2018 and 629.0 mm for 2019, and the water deficit occurs from July to September in 2018, in the total amount of 132.8 mm. Additionally, water deficit in 2019 occurred only in July and August with the total amount of 132.7 mm.

Excess water occurs for the most part during the winter months and at the beginning of spring ending with March in 2018 with total amount of 209.3 mm, while during 2019, ending with May and amounts to a total of 371.5 mm (Figure 8-9).

Table 4. Water balance in soil according to Thornthwaite for Maksimir, year 2018 and 2019

2018	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	SUM
Precipitation (mm)	56.7	87.5	72.2	65.8	68.7	127.8	85.2	40.7	59.0	88.6	80.4	21.0	958.8
Temperature (°C)	5.2	0.2	5.2	16.1	19.5	21.4	22.5	23.7	17.7	13.7	7.9	2.7	11.9
pet. kor. (mm)	10.6	0.1	13.5	75.1	112.4	131.0	141.7	140.5	79.4	49.8	19.1	3.9	777.1
R (mm)	100.0	100.0	100.0	90.7	47.0	43.9	0.0	0.0	0.0	38.8	100.0	100.0	720.4
set (mm)	10.6	0.1	13.5	75.1	112.4	131.0	129.1	40.7	59.0	49.8	19.1	3.9	644.3
m (mm)	0.0	0.0	0.0	0.0	0.0	0.0	12.6	99.8	20.4	0.0	0.0	0.0	132.8
v (mm)	46.1	87.4	58.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	17.1	209.3
2019	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	SUM
Precipitation (mm)	35.8	25.1	36.8	81.1	147.7	70.8	76.8	56.7	150.1	42.3	179.2	98.1	83.4
Temperature (°C)	1.5	4.8	9.5	12.4	13.7	23.8	22.9	23.5	17.2	13.2	9.2	4.6	11.9
pet. kor. (mm)	1.9	10.1	32.9	52.8	69.3	152.3	145.4	139.3	76.8	47.9	24.3	8.7	761.7
R (mm)	100.0	100.0	100.0	100.0	100.0	18.5	0.0	0.0	73.3	67.7	100.0	100.0	859.5
set (mm)	1.9	10.1	32.9	52.8	69.3	152.3	95.3	56.7	76.8	47.9	24.3	8.7	629.0
m (mm)	0.0	0.0	0.0	0.0	0.0	0.0	50.1	82.6	0.0	0.0	0.0	0.0	132.7
v (mm)	33.9	15.0	3.9	28.3	78.4	0.0	0.0	0.0	0.0	0.0	122.5	89.4	371.5

Note. pet.kor.-potential corrected evapotranspiration; R-water reserve in the soil; set-real evapotranspiration; m-lack of water; v-surplus water.

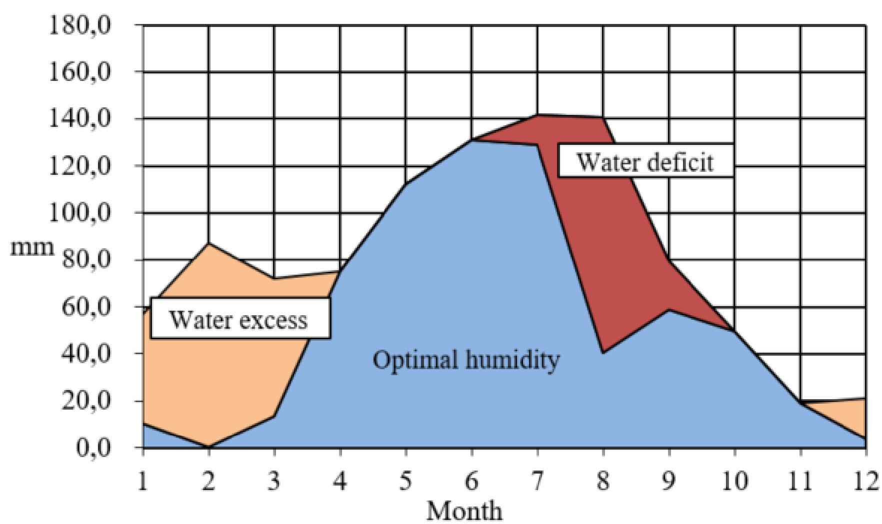

Figure 8. Soil water balance according to Thornthwaite for Maksimir, 2018

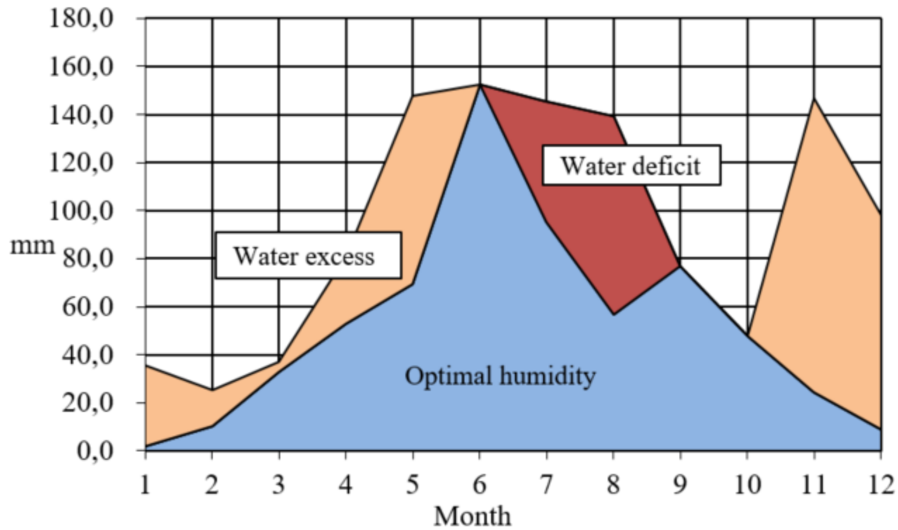

Figure 9. Soil water balance according to Thornthwaite for Maksimir, 2019

3.1.7. Walter's climate diagram

Walter's climate diagram can be used for an overview of the annual course of air temperature and precipitation. Among other things, it shows the mutual relationship between the mean monthly air temperature and the amount of precipitation on the basis of a long-term series of measurements.

The average values of the sum of precipitation and mean monthly temperatures by month are shown on the climate diagram for the year 2018 and 2019 (Figure 11-12). Based on the course of the precipitation and temperature curve, it can be concluded that the precipitation curve crosses the temperature curve in August, so there is a dry period on average during August. During 2019, precipitation curve also crosses the temperature curve in August which is resulted with drought. In June precipitation curve only approaches the temperature curve but there is no dry period on average.

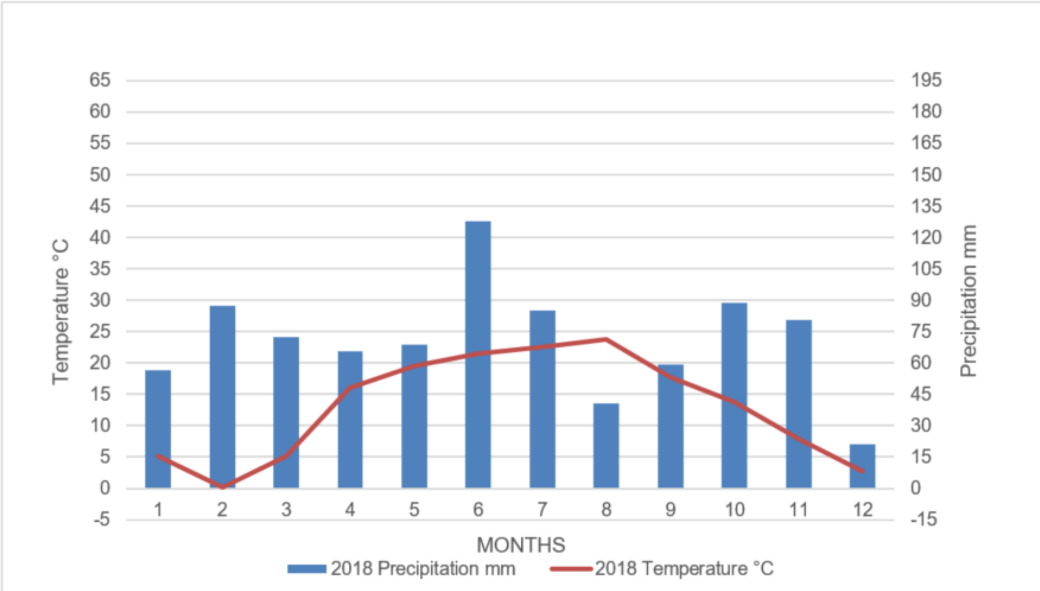

Figure 10. Presentation of the annual course of air temperature and amount of precipitation using Walter's climate diagram, Zagreb-Maksimir, 2018.

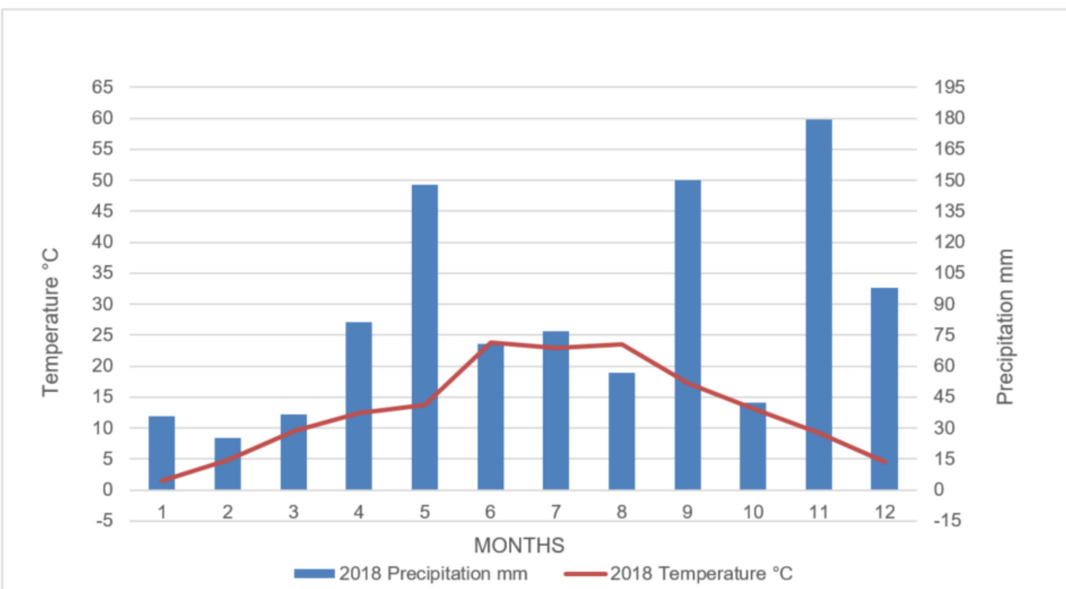

Figure 11. Presentation of the annual course of air temperature and amount of precipitation using Walter's climate diagram, Zagreb-Maksimir, 2019.

3.1.8. Soil characteristics

The soil characteristics of the experimental field, from 0-30 cm soil layer prior to experiment are shown in Table 5 and 6. Based on these data the fertilizing recommendation dosages were formulated.

Table 5. Chemical soil characteristics of the experimental field before the fertilization

Year	Depth	pH		Humus	N_{min}	NH_4^+-N	NO_3^--N	P_2O_5	K_2O
	cm	H_2O	KCl	%	kg ha^{-1}	mg kg^{-1}		mg 100 g^{-1} of soil	
2018	0-30	5.47	4.21	1.62	37.39	6.49	2.35	16.68	21.63
2019	0-30	5.26	3.93	1.56	38.21	4.78	4.25	14.13	18.53

Table 6. Chemical characteristics of total elements in the soil

Year	N	P	K	Ca	Mg	Mn	Fe	Pb	Cr	Co	Cd
	%					mg kg^{-1}					
2018	0.14	0.09	0.19	0.39	0.63	1142.46	35995	25.51	41.66	19.10	0.51
2019	0.13	0.09	0.19	0.39	0.60	1177.67	33051	18.25	39.69	19.86	0.45

Soil pH in KCl was 4.21 in 2018 and 3.93 in 2019, which is very acid reaction of the soil according to Thun (1955). In addition, the soil is poor in humus and has an average supply of available phosphorus and potassium.

In the both vegetation years 2018 and 2019, the test crops was maize (*Zea mays* L.) hybrid P 0725, FAO vegetation group 570. Soybean (*Glycine max* L.) was the previous crop in the vegetation season of year 2017. During vegetation period all management practices measures have been implemented (cultivation, weed and pest control).

3.1.8.1. Maize (*Zea mays* L.)

Maize is one of the most important cereal crops worldwide because, as a C4 plant, it has a high yield potential and an efficient photosynthetic capacity that enables effective CO_2 assimilation (Mengel, 1972; Sigurnjak et al., 2017; Herak Ćustić et al., 2025). It thrives best on deep, loose and well-drained soils, especially on medium-heavy and loamy soils that are rich in organic matter and have a neutral to slightly acidic pH value. For optimum maize cultivation, attention must be paid not only to the most important macronutrients (N, P and K) but also to micronutrients such as zinc (Zn). The plant is particularly sensitive to P deficiency in the early stages of development and has a high K requirement during panicle formation and the fertilization phase. Maize also has a high water requirement and reacts positively to the use of organic fertilizers (Herak Ćustić et al., 2025).

The maize hybrid P0725 (Figure 13), used in this experiment, which is classified in FAO maturity group 570, is a medium-late to late maturing variety that is suitable for both grain and silage production. It has proven good drought tolerance and yield stability in various environments. P0725 is characterized by tall plants with upright leaves, strong initial vigor and remarkable green retention, showing high resilience under stress conditions. For optimal results, it is recommended to plant this hybrid at a density of up to 60 000 plants per hectare and at appropriate spacing to support its robust growth and maximize yield potential. In both experimental years, basic tillage and soil preparation for sowing, plowing to a depth of 25–30 cm, spreading, fertilization and top dressing were carried out according to the established research methodology (Pioneer, 2025).

Figure 12. Miaze hybrid Pioneer 0725 (source: Pioneer, 2025)

For the first year of the experiment, soil was prepared by plowing the crop residues during November 2017. Tillage was done to 30 cm depth on 14 December 2017. Pre-sowing preparation and fertilization follows on 25 April 2018. Total amount of N, 140 kg ha^{-1} was applied to the soil on all treatments except on treatment with NPK+CAN where only 50% of total N was applied and other 50% was added to the soil during top dressing with CAN. Maize sowing has been done to 3-5 cm depth on 27 April 2018. During maize growing season weed protection was carried out by herbicide Lumax (S-metolachlor, atrazine and mesotrione) on 3 May in the amount of 2.5-3.5 l ha^{-1}. Cultivation with top dressing of CAN fertilizer (rest of 50% of total N) was added and performed during vegetative phase V5 or five fully developed leaves on 29 May. Harvest was done on 28 September 2018 (Figure 14).

Figure 13. Fertilization, sowing, maize and harvest (source: Šatvar Vrbančić, 2018)

During second year of the experiment, soil preparation starts with plowing of the crop residues in November 2018. Tillage to 30 cm depth was done on 20 December 2018. Pre-sowing preparation and fertilization follows on 10 April 2019. Same as during 2018, fertilization methodology was the same in year 2019. Maize was sown to 3-5 cm depth on 3 May and weed protection was carried out by herbicide Koban T (pethoxamide and terbuthylazine) in the amount of 3-3.4 l ha^{-1} and Deherban (dichlorophenoxyacetic acid) in the amount of 1.25 l ha^{-1} on 1 June 2019. Cultivation with top dressing was performed on 13 June during vegetative phase V5 or five fully developed leaves and maize harvest occurred on 10 October 2019 (Figure 15).

Figure 14. Fertilization, sowing, maize and harvest (source: Šatvar Vrbančić, 2019)

3.1.9. Product composition, sampling and analysis

Products that were used in the experiment were taken from AD plant Bojana in Čazma (Figure 16), Croatia (45°44'17, S; 16°39'05, I). Solid fraction of digestate (SFD) and liquid fraction of digestate (LFD) were sampled and collected at AD plant while liquid cattle manure (LCM) was collected from cattle farm (Figure 17) close to the AD plant.

Figure 15. Anaerobic digestion plant Bojana, Čazma (source: Šatvar Vrbančić, 2018)

Figure 16. Anaerobic digestion plant Bojana, Čazma (source: Google maps, 2025)

Product sampling and characterization were done two times. At first sampling, before fertilization, all products were collected from the AD plant to determine the required application rate for the test crop, while respecting the legal limits imposed by the Nitrates Directive and good agricultural practice (91/676/EEC). The day before the fertilization, products were sampled again and analyzed to determine nutrient content applied to the field.

The AD plant (Bojana Čazma) is operational since October 2014 and localized in a region characterized by agriculture and intensive cattle farming. The treatment capacity is 85 000 t y^{-1} and 28 000 MWh of electricity produced yearly under thermophilic digestion. The plant receives LCM from farmers in a radius of 10 km, and yearly 55% of cattle manure and 42% of maize silage is co-digested (Consult Are, 2024). SFD and LFD were obtained after screw press mechanical separation. Both products were collected from mixed storage tanks while LCM from a nearby cattle farm from non-mixed storage. All products were collected in polyethylene sampling bottles (3 L) and stored at 4°C until chemical analyses.

The physico-chemical characteristics of cattle manure, the SFD and LFD are shown in Table 7. As it is seen, the amount of extractable nutrients is higher for SFD and LFD in both years than for LCM. Also, there is a difference in dry matter content among products.

Table 7. Physico-chemical characterization of liquid cattle manure (LCM), solid fraction of digestate (SFD) and liquid fraction of digestate (LFD) per year

Parameters	**LCM**	**SFD**	**LFD**	**LCM**	**SFD**	**LFD**
Year	**2018**			**2019**		
Dry matter (%)	8.9	20.3	4.9	9.3	28.8	5.4
Organic matter (g kg^{-1})	71.8	87.3	69.5	77.6	88.1	69.6
Organic carbon (g kg^{-1})	40.2	48.9	38.9	43.5	49.3	39.0
pH	6.6	8.7	7.7	7.0	8.9	7.7
EC (mS cm^{-1})	13.0	1.3	15.2	13.4	1.5	17.7
N total (g kg^{-1}) FW	4.1	12.7	8.1	3.9	6.9	4.0
NH_4-N (g kg^{-1}) FW	0.6	0.6	0.8	1.3	1.3	1.7
N organic (g kg^{-1}) FW	3.5	12.1	7.3	2.6	5.6	2.3
P total (g kg^{-1}) FW	2.01	3.37	1.16	0.67	2.19	0.68
K total (g kg^{-1}) FW	3.46	2.78	3.53	2.79	2.61	3.33
Ca total (g kg^{-1}) FW	4.41	2.54	1.85	4.39	3.00	1.08
Mg total (g kg^{-1}) FW	0.75	1.40	0.93	0.93	1.59	0.55
Fe total (mg kg^{-1}) FW	114.32	193.51	133.66	83.07	196.25	79.48
Mn total (mg kg^{-1}) FW	15.75	83.98	12.44	23.63	37.18	21.76
C/N total	9.80	3.85	4.80	11.15	7.14	9.75
C/N organic	11.49	4.04	5.33	16.73	8.80	16.96
N/P	2.04	3.77	6.98	5.82	3.15	5.88
NH_4-N/N total	0.15	0.05	0.10	0.33	0.19	0.43
N organic/N total	0.85	0.95	0.90	0.67	0.81	0.58
HEAVY METALS (DW)						
Maximum permitted concentrations (MPC) are prescribed by the Ordinance on the Protection of Agricultural Land from Pollution (NN 71/19)						
Zn total (mg kg^{-1}) DW	175.0	76.7	229.0	215.0	78.5	263.0
Cu total (mg kg^{-1}) DW	231.0	102.0	292.0	61.4	45.8	139.0
Ni total (mg kg^{-1}) DW	2.17	2.25	0.16	8.84	5.88	12.90
Pb total (mg kg^{-1}) DW	<1.0	<1.0	2.67	<0.1	<0.1	<0.1
Cr total (mg kg^{-1}) DW	47.0	40.2	45.2	79.8	56.1	64.5
Cd total (mg kg^{-1}) DW	<0.1	<0.1	0.44	0.39	<0.1	0.15
Hg total (mg kg^{-1}) DW	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Note. LCM-liquid cattle manure; SFD-solid fraction of digestate; LFD-liquid fraction of digestate, FW – fresh weight; DW – dry weight

3.1.10. Sampling and analysis

3.1.10.1. Soil sampling

Soil samples were taken from each experimental plot before fertilization treatments (16 April 2018 and 16 April 2019) and then three times during different maize growth stages (vegetative stage V4 or four fully developed leaves (21 May 2018 and 10 June 2019); reproductive stage R5 or dent stage (28 August 2019 and 27 August 2019); reproductive stage R6 or after physiological maturity stage (25 September 2018 and 01 October 2019)) (Abendroth et al., 2011). Homogenized soil samples were taken at three soil depths (0-30 cm, 30-60 cm and 60-90 cm) using an auger. The samples were collected in polyethylene sampling bags and transported from the field to the laboratory of Department of Plant Nutrition, Faculty of Agriculture (Zagreb, Croatia) for further analysis. As well part of samples were analysed in the laboratory of Ghent University Faculty of Bioscience Engineering in Belgium. Each soil sample was divided into two parts, wet soil for mineral N (NO3-−N and NH4+−N) determination (0-30, 30-60 and 60-90 cm) were cold stored, while the second part of the soil was air-dried at room temperature (25°C) and analyzed for pH, total P2O5 and K2O, other macro and microelements (0-30 cm).

3.1.10.2. Plant sampling

Maize samples were taken three times during the vegetative growth (vegetative stage V4 or four fully developed leaves – May/June; reproductive stage R5 or dent stage – August and harvested in reproductive stage R6 or after physiological maturity stage – September/October) as already mentioned in section 3.1.10.1. (Abendroth et al., 2011). During first and second sampling, from each plot 12 maize plants were randomly harvested, while at harvest time two middle rows of each plot were taken. Plants were cut at ground level and taken to the laboratory, chopped and homogenously mixed for analysis. From this mixture 500 g of sample was oven-dried at 105°C for determination of the dry matter (DM) (%) content. The dry samples were grinded and prepared for chemical analysis of all macro and microelements.

Additionally, during V4 and R5 stage of maize growth, content of nitrates in the whole plant was done.

3.1.10.3. *Digestate fractions, soil and plant measurements (or chemical analysis)*

Analysis of all materials were analysed accordingly: DM was determined as a remaining mass after 48 h drying at 105°C. Total N was determined using Kjeldahl destruction (HRN ISO 11261:2004) (Bremner, 1964), and ammonium-N ($NH_4^+{-}N$) using KjeltecTM 8100 distilling unit (Bremner, 1964) after addition of MgO to the sample, and subsequent titration. As for other parameter analysis, organic matter (OM) was measured after incineration of the samples during 3 h at 550°C in a muffle furnace, where the loss of mass on ignition was addressed as the OM. Organic carbon (OC) was determined by conversion factor (European Parlament, 2019), as follows:

$$\text{organic carbon } (C\_org\ ) = \text{organic matter } \times 0.56$$

EC and pH were determined by using a Mettler Toledo conductivity EL30/EL3 electrode and a Mettler Toledo pH-meter EL20/EL2, respectively. For liquid samples, conductivity (EC) and reaction (pH) measurements were performed directly in original sample, while solid samples were equilibrated in deionized water for 1 h at a 10:1 liquid to dry sample ratio. Afterwards filtration of suspension was performed, and pH and EC were measured from the filtered solution. Organic N (ON) was calculated by subtraction of $NH_4^+{-}N$ from the total N. After aqua regia microwave digestion (5 ml HNO_3 and 15 ml HCl) on dry sample, total phosphorus (P), sulfur (S), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu) and Zn were measured: P with spectrophotometer Evolution 60S UV-Visible Spectrophotometer, K with flame photometer JENWEY PFP 7, and other macro and microelements with atomic absorption spectrometer Solaar M5 Series (AOAC, 2015).

3.1.10.4. <u>Soil analysis</u>

The moisture content was determined by weight loss after drying the soil sample to a constant weight at 105 °C for at least 24 h. pH was determined by using a Mettler Toledo pH-meter. Total N content in soil was determined using the Kjeldahl destruction method (HRN ISO 11261:2004). Nitrate-N (NO_3^-−N) and ammonium-N (NH_4^+−N) in soil were extracted according to phenoldisulfonic acid method (Keeney and Nelson, 1982) and Nessler method (JDPZ, 1966) respectively, and subsequently analyzed using a spectrophotometer Evolution 60S UV-Visible Spectrophotometer. Available P and K were determined with ammonium lactate (AL) method (Egnér et al., 1960). Afterwards P was analysed on spectrophotometer Evolution 60S UV-Visible Spectrophotometer and K on flame photometer JENWEY PFP 7 (AOAC, 2015).

3.1.10.5. <u>Plant analysis</u>

The above ground plant samples were collected in the field and weighed for determination of the fresh weight (FW) biomass yield. After oven-dried at 105°C for determination of the dry weight (DW) content. The dry samples were grounded and afterwards analysed. Total N content in plant was determined using the Kjeldahl destruction method (HRN ISO 11261:2004). After microwave digestion (9 ml HNO_3 and 1 ml H_2O_2) on dry sample, total P, S, K, Ca, Mg, Cu and Zn were measured: P with spectrophotometer Evolution 60S UV-Visible Spectrophotometer, K with flame photometer JENWEY PFP, and other macro- and microelements with atomic absorption spectrometer Solaar M5 Series (AOAC, 2015).

3.1.11. Calculations

Apparent N recovery (ANR) and N fertilizer replacement value (NFRV) were calculated as follows (Sigurnjak et al., 2017):

$$\text{Apparent N recovery (ANR)} = \frac{\text{N uptake}_{\text{TREATMENT}}\ (\text{kg ha}^{-1}) - \text{N uptake}_{\text{CONTROL}}\ (\text{kg ha}^{-1})}{\text{Total N applied}_{\text{TREATMENT}}\ (\text{kg ha}^{-1})} \qquad \text{(Eq. 1)}$$

$$\text{N fertilizer replacement value (NFRV; \%)} = \frac{\text{ANR}_{\text{BIO-BASED TREATMENT}}}{\text{ANR}_{\text{REFERENCE}}} \times 100 \qquad \text{(Eq. 2)}$$

where “control” is unfertilized, “treatment” contains one of the tested materials (LCM, SDF, LFD, SFD+MF, LFD+MF) and “reference” is a mineral fertilizer (MF).

Nutrient and mass balance was calculated as the difference between nutrient input from fertilization and nutrient removal by crop uptake, representing an apparent mass balance approach commonly used to evaluate nutrient dynamics in agricultural systems (Oenema et al., 2003; FAO, 2006; FAO, 2025).

$$\text{Nutrient balance} = \text{Fertilizer input} - \text{Plant uptake} \qquad \text{(Eq. 3)}$$

3.2. LABORATORY EXPERIMENT – NITROGEN INCUBATIONS

3.2.1. Experiment setup

A laboratory experiment was carried out in the poly vinyl chloride (PVC) tubes with a diameter of 4.6 cm and 25 cm in length for 120 days. Different fertilization treatments were mixed with the soil and fertilizers were applied at a rate of 170 kg total N ha^{-1}. Same fertilizers used in the field experiment were used in the laboratory experiment and they consisted of synthetic fertilizer (NPK + CAN), liquid cattle manure (LCM), solid fraction of digestate (SFD), liquid fraction of digestate (LFD) and a mixtures of digestate fractions with mineral fertilizer (SFD + NPK and LFD + NPK). Additionally 140 kg of total N was used for fertilization. As for the control treatment (C) no fertilizer was added in the soil. The soil was brought to a bulk density of 1.4 g cm^{-3} by compacting the mixture to a height of 10 cm. The treatment with mineral N fertilizer (i.e. NPK (15% N) and CAN (27% N) in ratio 50:50) was used as a reference for the conventional fertilizer. All fertilizers were applied on the top of a surface and than carefully mixed. The moisture content of the incubated soil was adjusted to 50% of water filled pore space (WFPS) by adding deionized water.

3.2.2. Soil preparation and analysis

The soil that was used for the incubation experiment was collected on 24 April, 2018 from the surface layer (0-30 cm) of a same field that was used for the field experiment (Maksimir, Croatia, 45°49'39,S; 16°02'02,E). The soil texture is classified as silt-loam soil and contains 15.4% sand, 67.5% silt and 17.1% clay. Maize as a field test crop was cultivated in 2018. The soil sample was taken before fertilization and sowing of the maize for the moisture, total and mineral N content determination in the laboratory as well as for the incubation experiment. The values of dry matter, total N, NO_3^-−N, NH_4^+−N, organic matter (OM), organic carbon (OC) are explained and described in the section 3.1.10.1. and 3.1.10.2. The characteristics of the tested soil are shown in the Table 8.

Table 8. The characteristics of the tested soil

Depth cm	pH_{H2O}	pH_{KCl}	Humus %	N total g kg^{-1}	NH_4^+-N g kg^{-1}	NO_3^--N g kg^{-1}
0-30	5.47	4.21	1.62	1.36	4.56	10.64

3.2.3. Fertilizer collection and analysis

The tested fertilizers were collected from a biogas plant Bojana, Čazma. All informations about the biogas plant and its operational system is explained in the section 3.1.9.

Digestate fractions (SFD and LFD) with LCM were characterized in the Table 9. The values of DW, OM, total N, OC, NH_4^+−N, and NO_3^-−N were determined as described in the section 3.1.10.1.

Table 9. Characterization of the bio-based materials on fresh weight basis

Parameters	**LCM**	**SFD**	**LFD**
Dry matter (%)	8.9	20.3	4.9
Organic matter (g kg^{-1})	71.8	87.3	69.5
Organic carbon (g kg^{-1})	40.2	48.9	38.9
N total (g kg^{-1})	3.2	6.2	3.4
NH_4-N (g kg^{-1})	0.6	0.6	0.8
N organic (g kg^{-1})	2.6	5.6	2.6
C/N total	9.80	3.85	4.80
C/N organic	11.49	4.04	5.33
NH_4-N/N total	0.19	0.10	0.24
N organic/N total	0.81	0.90	0.76

Note. LCM-liquid cattle manure; SFD-solid fraction of digestate; LFD-liquid fraction of digestate.

3.2.4. Laboratory incubation and sampling

Before the experiment was set up, from 5 May to 15 May 2018, the soil for the incubations was air-dried in the greenhouse, afterwards mixed and pre-incubated at 35% water filled pore space (WFPS) for one week at 25 °C in the dark (Figure 18).

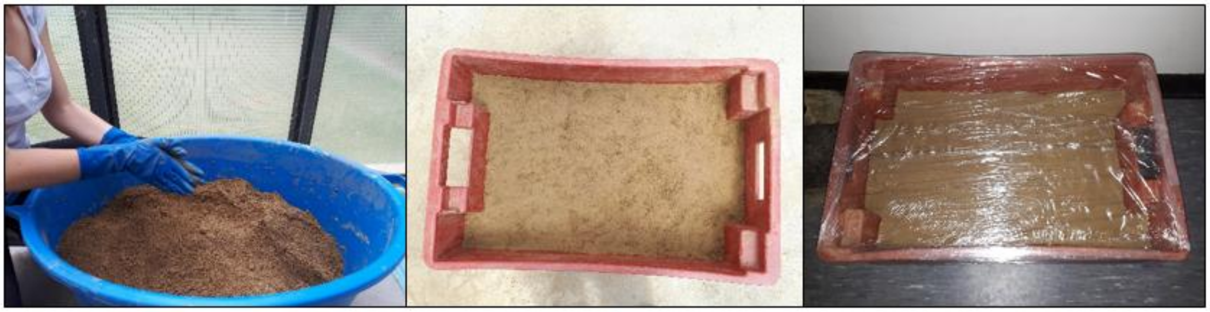

Figure 17. Pre-incubation of the soil (source: Šatvar Vrbančić, 2018.)

At the start of the incubation experiment (Figure 20), synthetic fertilizer (NPK + CAN), LCM, SFD, LFD, a mixture of SFD+NPK and a mixture LFD+NPK were thoroughly mixed with 254 g of pre-incubated soil (equivalent to 237 g of air-dried soil) at a rate of 170 kg of total N ha^{-1}. Homogenous mixture was placed into PVC incubation tubes with a diameter of 4.6 cm and 25 cm in length. The soil was brought to a bulk density of 1.4 g cm^{-3} by compacting the mixture to a height of 10 cm. The treatment with synthetic N fertilizer (i.e. NPK (15% N) and CAN (27% N) in ratio 50:50) was used as a reference for the conventional fertilizer. For the control, bare soil was used. Distilled water was added to each tube to achieve 50% WFPS and after that covered with a single layer of pin-holed gas permeable parafilm to minimize water loss whilst allowing air exchange. Each tube was weighted, and the moisture content was monitored every two weeks during the incubation period by weighing the tubes and maintaining them at 50% WFPS by adding distilled water when needed. Temperature of growing chamber was set to 18 °C (Figure 19). Three separate replicates of seven treatments and the control were analysed at day 20, 40, 60, 80, 100 and 120 by removing intact tubes.

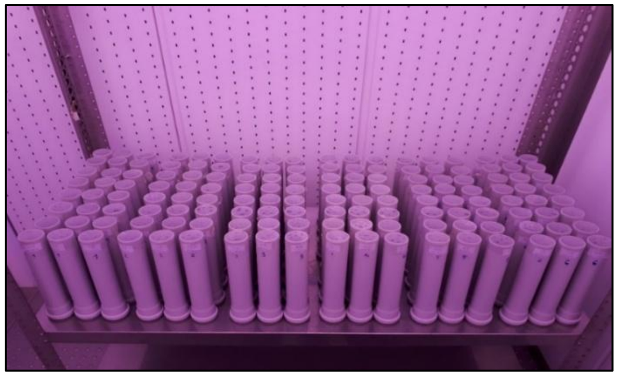

Figure 18. Incubation set in the growing chamber (source: Šatvar Vrbančić, 2018)

The mineral N in the control treatment was determined again at day 0 in order to include any effects of soil air-drying and re-wetting. The soil was removed from the tubes, mixed thoroughly, and analyzed for soil NO_3^-−N and NH_4^+−N (see section 3.1.10.3. and 3.1.10.4. for the description of the analysis). It should be noted that under these experimental conditions the volatilization of ammonia is considered negligible, as the materials and soil were homogeneously mixed and there was no air flow at the soil surface during incubation (de la Fuente et al., 2010; Alburquerque et al., 2012a from Sigurnjak et al., 2017).

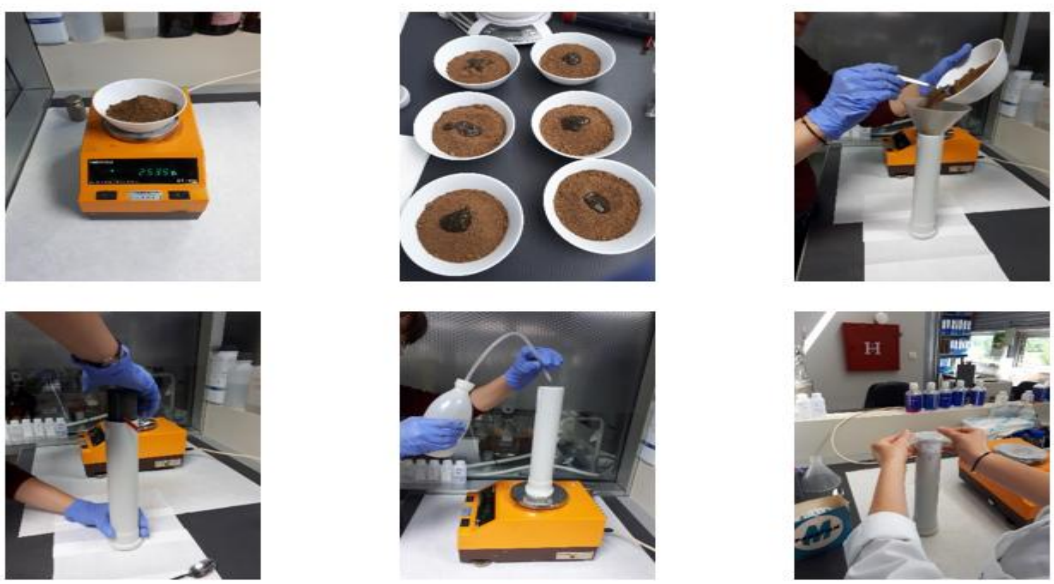

Figure 19. Experiment setup of N incubation experiment for determination of N release and N mineralization (source: Šatvar Vrbančić, 2018).

3.2.5. Calculations and data analysis

The net N release ($N_{rel,net}$) was calculated as the difference between the mineral N measured in the amended soil minus the mineral N measured in the blank (i.e. unamended soil), calculated according to De Neve & Hofman (1996), as follows:

$$N_{rel,net}\,(\%) = \frac{([NO3^{-}-N,treatment]-[NO3^{-}-N,control])+([NH4^{+}-N,treatment]-[NH4^{+}-N,control])}{Ntotal\ applied} \times 100$$

Eq. 4

Net N mineralization ($N_{min,net}$) in the product-amended treatments and unamended control was calculated by subtracting the inorganic N content of the treatment at day 0 from the measured inorganic N in the sample at all subsequent measurements, as follows (Sigurnjak et al., 2017):

$$N_{min,net}\,(t;\ \%\ total\ N) = (N_{rel,net}\,(t) - N_{rel,net}\,(t{=}0))$$

Eq. 5

3.3. STATISTICAL ANALYSIS

3.3.1. Statistical analysis of field experiment

An analysis of variance (ANOVA) was performed separately for each year (2018 and 2019) to evaluate the physicochemical properties of the soil and plant samples. The aim of the analysis was to evaluate and compare the nutrient contents (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, Ni, Pb, Cr, Co, Cd and nitrates) between the different fertilization treatments.

An analysis of variance (ANOVA) was performed to assess the effects of various factors on measured yields and physicochemical analyses of soil and plant samples. The ANOVA model included fixed effects for year, fertilization treatment, and phenophase (where applicable) and the random effect of replication.

Before ANOVA, assumptions of normality and homogeneity of variances were checked using Shapiro-Wilk and Levene's tests, respectively.

The significance level for all statistical tests was set at $\alpha = 0.05$. When significant main effects or interactions were detected, Tukey's HSD (Honestly Significant Difference) or post-hoc test was applied to determine specific differences between treatment means.

Furthermore, soil and plant nutrient concentrations, apparent nutrient and heavy metal balances were calculated as the difference between fertilizer input and plant uptake for the entire experimental period (2018-2019). For statistical evaluation, balances summed over both years were analyzed using one-way analysis of variance (ANOVA) to assess differences among fertilization treatments. When significant treatment effects were detected, Tukey's HSD post hoc test was applied to identify differences between treatment means. Prior to analysis, assumptions of normality and homogeneity of variances were verified.

All statistical analyses were performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL).

3.3.2. Statistical analysis of laboratory experiment

The analysis of variance (ANOVA) with repeated measures was performed using the MIXED procedure in SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). In the mixed model analysis, an autoregressive covariance structure of order 1 [AR(1)] was applied, assuming that the correlation between repeated measurements decreases as the time interval between them increases, which is appropriate for longitudinal data. Also, model included the effects of treatment (unfertilized control (C); mineral fertilizer (NPK+CAN); liquid cattle manure (LCM); solid fraction of digestate (SFD); liquid fraction of digestate (LFD); mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK) and mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK), time (used as repeated measures), and treatment × time interaction on measured variables (NH_4^+, NO_3^-, N_{min} and N_{rel}).

Tukey's Honest Significant Difference post hoc test was performed for partitioned F-tests (SLICE option) to examine the significance of treatments differences within time, and time differences within treatments.

3.3.3. Statistical analysis and comparison between field and laboratory data

3.3.3.1. Correlation

Pearson's correlation analysis was performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL) to examine the relationships between different forms of mineral nitrogen (NH_4^+, NO_3^-, and Total N) obtained from laboratory and field experiments. Correlation coefficients (r) and their corresponding significance levels (p) were calculated to assess the strength and direction of associations between variables. The analysis was conducted separately for laboratory and field data, as well as between laboratory and field datasets, to evaluate potential linkages under controlled and natural conditions. The significance of correlations was interpreted at the 0.05 and 0.01 probability levels.

3.3.3.2. Regression

Simple linear regression analysis was performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL) to evaluate the effect of temperature on mineral nitrogen forms (NH_4^+ and NO_3^-) in the field experiment. Temperature was used as the independent (predictor) variable, while NH_4^+ and NO_3^- concentrations were the dependent variables. The strength of the relationship was assessed using the correlation coefficient (R), the coefficient of determination (R^2), and the F-test from the analysis of variance table. The regression coefficient (B) indicated the direction and magnitude of temperature effects on nitrogen forms, with statistical significance evaluated at the 0.05 probability level.

4. RESULTS

4.1. SOIL CHARACTERISTICS

The soil characteristics of the experimental field, from 0-30 cm soil layer prior to experiment are explained and shown in Table 5 and 6 (see section 3.1.8.).

The effects of solid and liquid digestates on the chemical properties of the soil, the nutrient capacity of the plants and the degree of contamination of the soil with inorganic pollutants (heavy metals) were determined on the basis of the results of soil chemical analyses carried out in eight soil sampling during two vegetation periods of maize growth and development.

4.1.1. Soil pH

Table 10 shows the values of the soil pH value in the water, in the arable layer from 0-30 cm of the soil. The soil pH value determined in 1M KCl are also given. According to Thun (1955), the experimented soil belongs to the soils with a strongly acid reaction in the arable and sub-arable layer. The table contains soil pH measurements taken under different treatments on two sampling dates: September 25, 2018 and October 1, 2019 (both years at the end of the experiment). The soil pH was measured both in water (pH_{H_2O}) and in a 1M potassium chloride solution (pH_{KCl}). At the beginning, the soil pH was 5.47 in H_2O and 4.21 in KCl in 2018 and 5.26 in water and 3.93 in KCl in 2018.

After one year (2018), slight fluctuations in pH were observed between treatments, with values ranging from 5.29 to 5.52 in water and from 3.98 to 4.17 in KCl. In 2019, pH values were generally slightly lower compared to the first measurements, but varied between treatments. The lowest pH drop in pH_{KCl} occurred in LFD and LFD+NPK treatment.

The pH measured in water in 2019 ranged from 5.43 to 5.63, while the pH_{KCl} fluctuated between 4.04 and 4.29. Compared to 2018, the pH value increased slightly in all treatments compared to the initial soil. The greatest pH change again occurred in the LFD and LFD+NPK treatments.

Table 10. Soil pH reaction through vegetation before and at the end of the experiment

	pH H_2O	pH 1M KCl	pH H_2O	pH 1M KCl
Initial soil	5.47	4.21	5.26	3.93
	25 September 2018	25 September 2018	01 October 2019	01 October 2019
C	5.39	4.02	5.43	4.04
NPK+CAN	5.34	4.03	5.63	4.19
LCM	5.52	4.09	5.65	4.18
SFD	5.41	3.98	5.64	4.22
LFD	5.30	4.17	5.54	4.25
SFD+NPK	5.29	4.06	5.45	4.04
LFD+NPK	5.33	4.15	5.50	4.29

Note. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.1.2. Soil Nutrient and Heavy Metal Dynamics Under Different Fertilizer Treatments (2018–2019)

The results of the chemical analyses summarized in Table 11 (2018) and Table 12 (2019) showed that total nitrogen (N) content, evaluated according to the Woltmann classification, provided a good N supply in the arable soil layer (0–30 cm) across all treatments, with only minor treatment-related fluctuations during the growing seasons. In 2018, significant differences appeared only during the second sampling, when the C and NPK+CAN treatments showed slightly higher total N values compared with LCM and LFD, while in 2019 the highest total N values at the second sampling were found in LCM and SFD, with the control showing the lowest levels.

Mineral N (Nmin) dynamics followed a clear seasonal pattern in both years. In 2018, the highest values at the first sampling were recorded in NPK+LFD, followed by NPK+CAN and LCM, whereas all treatments experienced a strong decline in the second sampling, with NPK+CAN maintaining the highest value; by the third sampling, Nmin increased again, and NPK+LFD regained the highest value. A similar trend was observed in 2019, where LFD, NPK+SFD and NPK+CAN had significantly higher Nmin concentrations than the control at the first sampling, NPK+CAN and NPK+LFD dominated at the second sampling, and NPK+CAN maintained the highest Nmin content at the final sampling of the season.

Regarding total phosphorus (P), no statistically significant differences between treatments were detected in the first two samplings of either year. However, in 2018 the LCM treatment reached the highest total P content at the third sampling, while C and NPK+CAN had the lowest values, and in 2019 the highest total P contents in the final sampling occurred in LFD, SFD+NPK and LFD+NPK, with LCM, SFD and C recording the lowest levels. Available P showed no significant treatment effects in either year.

Available P showed no significant differences between treatments in either year, aside from a notably higher level in LFD+NPK at the end of 2018.

Total potassium (K) also revealed year-specific behaviour. In 2018, the first sampling showed the highest total K in SFD+NPK and LFD+NPK and the lowest in NPK+CAN, while the second sampling showed LCM with the highest total K and SFD+NPK the lowest; by the third sampling, total K was lowest in LCM, LFD and SFD+NPK, and highest in NPK+CAN. In 2019, LCM had the highest total K in the first sampling and LFD+NPK the lowest, no differences appeared in the second sampling, while in the final sampling treatments C, SFD, SFD+NPK and LFD+NPK had the highest total K levels and LFD the lowest.

For available K, the highest concentration in 2018 was recorded in the LFD+NPK treatment, while the lowest was observed in the SFD treatment. In 2019, there were no significant differences among treatments.

Total calcium (Ca) remained constant at the initial soil concentration throughout both years, with no significant effects of fertilization, and total magnesium (Mg) also remained stable, except for slightly elevated values in SFD+NPK and LFD+NPK at the first sampling in 2018 and a relatively higher value in the control at the second sampling.

Total manganese (Mn) concentrations did not differ significantly between treatments in either year but remained stable relative to the initial level in 2018, while in 2019 they increased moderately compared with the initial value, suggesting an influence of seasonal conditions.

Total iron (Fe) showed no treatment effects in the first two samplings of 2018, although SFD exhibited the highest Fe concentration and NPK+CAN the lowest in the third sampling, whereas in 2019 no differences appeared at any sampling point, despite an overall seasonal increase compared with the initial Fe level.

For total zinc (Zn), significant differences occurred only at the first sampling of 2018, where LCM and SFD showed the highest values and SFD+NPK and LFD+NPK the lowest, while in 2019 no significant differences were found; in both years, Zn levels remained below the initial soil concentrations and consistently above the MPC without exceeding the starting values.

Total copper (Cu) displayed no differences in the first two samplings of 2018 but showed higher concentrations in C, SFD+NPK and LFD+NPK at the final sampling, while LCM and LFD had the lowest values; no significant differences appeared in 2019, and in both years Cu remained well below the MPC and close to initial soil concentrations.

Total nickel (Ni) differed only in the first sampling of 2018, where SFD showed the highest and SFD+NPK and LFD+NPK the lowest concentrations, and in 2019 only at the third sampling, where NPK+SFD had the highest and NPK+CAN the lowest values; in both years. Ni remained above the MPC due to high initial soil concentrations but did not increase substantially during the season.

Total lead (Pb) displayed no differences in 2018 except for the second sampling, where the control had the highest and SFD the lowest concentration; in 2019 no differences were observed, and in both years Pb remained safely below the MPC.

Total chromium (Cr) differed at the first sampling in both years SFD being highest and SFD+NPK and LFD+NPK lowest in 2018, and the control highest and LCM and NPK+SFD lowest in 2019 while no differences occurred later in either year. Although Cr was slightly above the MPC initially in 2018 and slightly below it in 2019, only the latter year showed a seasonal increase above both the initial value and the limit.

Total cobalt (Co) remained far below the MPC in all treatments and both years without significant treatment differences, aside from a minor increase in the second sampling of 2019.

Finally, total cadmium (Cd) levels remained well below the MPC in both years, with fluctuations occurring among treatments, including higher Cd concentrations in NPK+CAN in both years and the lowest values generally found in LCM and SFD, yet without any signs of excessive accumulation relative to initial soil conditions.

Table 11. Effect of different fertilizer treatments and element contents on the soil at different times of sampling during 2018

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
N total %	Initial soil	0.14±0.00						
	May	0.14±0.01	0.14±0.01	0.13±0.00	0.13±0.01	0.13±0.00	0.13±0.00	0.13±0.00
	August	0.13±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{b}	0.13±0.01^{ab}	0.12±0.00^{b}	0.12±0.01^{ab}	0.13±0.01^{ab}
	September	0.13±0.01	0.13±0.00	0.12±0.00	0.12±0.01	0.13±0.01	0.12±0.00	0.12±0.00
N min kg ha^{-1}	Initial soil	37.39±3.57						
	May	67.25±7.74^{d}	115.02±16.61^{ab}	84.81±16.07^{cd}	73.85±7.72^{cd}	89.79±6.24^{bcd}	96.16±9.84^{bc}	126.24±14.85^{a}
	August	16.26±2.30^{ab}	36.71±24.40^{a}	19.65±6.34 ^{ab}	12.84±1.91^{b}	26.15±3.65^{ab}	26.82±2.57^{ab}	22.47±2.75^{ab}
	September	30.82±2.98^{bc}	40.77±11.80^{ab}	28.82±2.96^{bc}	24.42±2.12^{c}	30.03±1.66^{bc}	41.26±5.68^{ab}	51.36±8.61^{a}
P %	Initial soil	0.09±0.00						
	May	0.08±0.00	0.08±0.00	0.08±0.01	0.08±0.00	0.09±0.01	0.08±0.00	0.08±0.00
	August	0.08±0.00	0.08±0.00	0.09±0.00	0.09±0.00	0.08±0.00	0.09±0.00	0.09±0.00
	September	0.08±0.00^{b}	0.08±0.00^{b}	0.09±0.01^{a}	0.09±0.00^{ab}	0.09±0.00^{ab}	0.09±0.00^{ab}	0.09±0.00^{ab}
P – avl* mg 100 g^{-1}	Initial soil	16.68±1.06						
	September	12.59±1.34	13.95±2.00	12.50±0.32	13.05±0.79	12.95±1.71	13.50±0.61	14.63±1.12
K %	Initial soil	0.19±0.01						
	May	0.22±0.01^{ab}	0.21±0.01^{b}	0.21±0.02^{ab}	0.22±0.02^{ab}	0.22±0.01^{ab}	0.24±0.01^{a}	0.24±0.00^{a}
	August	0.23±0.01^{ab}	0.22±0.01^{ab}	0.25±0.01^{a}	0.23±0.02^{ab}	0.24±0.01^{ab}	0.23±0.01^{ab}	0.22±0.01^{b}
	September	0.22±0.01^{a}	0.22±0.00^{a}	0.19±0.01^{c}	0.20±0.01^{bc}	0.20±0.01^{c}	0.20±0.01^{c}	0.20±0.01^{abc}
K – avl* mg 100 g^{-1}	Initial soil	21.63±1.46						
	September	17.00±1.39^{ab}	17.55±1.31^{ab}	17.83±1.91^{ab}	16.20±0.57^{b}	17.78±1.58^{ab}	17.78±1.58^{ab}	19.68±0.77^{a}
Ca %	Initial soil	0.39±0.00						
	May	0.41±0.02	0.37±0.01	0.39±0.03	0.38±0.02	0.38±0.01	0.40±0.02	0.41±0.02
	August	0.40±0.01	0.39±0.03	0.42±0.01	0.41±0.04	0.41±0.01	0.41±0.03	0.40±0.02
	September	0.40±0.02	0.40±0.02	0.39±0.03	0.41±0.01	0.39±0.03	0.39±0.02	0.41±0.02

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Mg %	Initial soil	0.63±0.01						
	May	0.65±0.00^{b}	0.65±0.02^{b}	0.65±0.01^{b}	0.64±0.02^{b}	0.64±0.01^{b}	0.72±0.01^{a}	0.72±0.01^{a}
	August	0.73±0.01^{a}	0.69±0.02^{abc}	0.68±0.01^{bc}	0.69±0.02^{abc}	0.70±0.01^{ab}	0.67±0.03^{bc}	0.66±0.03^{c}
	September	0.65±0.02	0.64±0.02	0.64±0.01	0.65±0.01	0.64±0.02	0.65±0.01	0.65±0.02
Mn g kg^{-1}	Initial soil	1.14±0.03						
	May	1.07±0.02	1.09±0.05	1.11±0.04	1.10±0.07	1.16±0.14	1.06±0.04	1.04±0.02
	August	1.03±0.03	1.16±0.12	1.20±0.10	1.15±0.09	1.10±0.03	1.13±0.07	1.15±0.07
	September	1.10±0.02	1.07±0.03	1.13±0.05	1.12±0.05	1.15±0.05	1.09±0.05	1.12±0.07
Fe g kg^{-1}	Initial soil	36.00±0.62						
	May	34.94±1.01	34.93±0.48	34.73±1.12	35.47±1.77	35.68±1.42	35.49±0.70	35.56±0.95
	August	36.33±1.21	36.54±1.20	36.30±0.66	36.31±0.93	36.28±0.80	36.88±0.33	36.44±0.31
	September	35.64±1.53^{ab}	35.15±0.68^{b}	36.97±0.85^{ab}	38.18±0.51^{a}	37.74±1.25^{ab}	38.02±1.79^{ab}	37.89±1.61^{ab}
Zn mg kg^{-1}	Initial soil	83.82±0.93						
	May	78.07±1.37^{ab}	79.19±1.42^{ab}	80.22±1.00^{a}	80.20±0.86^{a}	78.35±0.89^{ab}	76.74±1.79^{b}	76.30±1.26^{b}
	August	77.04±0.50	77.67±1.60	77.10±1.43	76.69±1.44	76.41±0.30	78.53±1.62	83.04±7.38
	September	81.15±4.60	78.57±1.48	80.89±1.13	82.31±0.81	82.47±1.03	78.91±1.45	78.88±1.89
Cu mg kg^{-1}	Initial soil	27.44±0.20						
	May	27.07±1.08	26.36±0.39	26.78±0.80	26.57±1.45	26.26±1.13	25.81±0.59	26.38±1.21
	August	26.20±0.68	26.59±1.39	26.76±1.22	26.15±0.76	27.32±2.75	26.81±0.78	27.77±1.56
	September	27.41±1.24^{a}	26.80±1.06^{ab}	23.99±0.36^{b}	24.45±1.34^{ab}	23.89±1.40^{b}	27.38±1.70^{a}	27.30±1.86^{a}
Ni mg kg^{-1}	Initial soil	32.05±0.23						
	May	31.19±1.31^{ab}	31.94±0.68^{ab}	31.78±0.75^{ab}	32.97±1.59^{a}	32.47±1.11^{ab}	30.17±1.39^{b}	30.28±0.52^{b}
	August	31.11±0.75	31.58±1.07	32.36±0.88	32.51±0.75	31.78±1.32	31.51±0.70	31.40±0.96
	September	32.42±0.85	31.65±0.28	31.82±0.64	31.40±0.71	31.45±0.58	31.61±1.30	32.24±1.59

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Pb mg kg^{-1}	Initial soil	25.51±0.76						
	May	25.86±1.67	27.27±1.76	26.61±2.12	27.72±1.01	27.46±1.79	27.87±3.62	29.45±0.73
	August	29.85±1.81^{a}	26.93±2.57^{ab}	24.18±2.71^{bc}	20.83±0.91^{c}	24.29±3.45^{bc}	25.77±2.52^{abc}	25.16±1.31^{abc}
	September	25.56±1.84	23.72±0.95	24.75±3.20	25.12±3.20	24.52±2.69	25.21±1.40	24.74±1.50
Cr mg kg^{-1}	Initial soil	41.66±0.68						
	May	40.47±0.36^{ab}	40.26±0.81^{ab}	41.07±2.12^{ab}	42.66±2.05^{a}	40.63±0.81^{ab}	39.23±1.04^{b}	38.81±0.76^{b}
	August	39.56±0.41	40.67±1.60	41.88±0.99	41.12±1.01	40.55±0.22	41.28±1.51	40.90±0.39
	September	40.78±0.56	40.76±1.26	40.18±0.42	41.76±0.49	41.00±1.22	41.15±1.10	40.46±0.55
Co mg kg^{-1}	Initial soil	19.10±0.37						
	May	17.99±0.90	18.42±0.28	18.48±0.86	18.33±0.74	18.82±2.18	18.22±0.64	17.78±0.86
	August	17.99±0.63	19.40±1.10	19.00±1.18	18.65±0.43	18.79±0.72	19.19±1.23	18.99±0.94
	September	18.78±0.99	18.18±0.92	19.14±1.16	19.28±1.05	18.69±0.51	18.66±0.89	18.76±1.46
Cd mg kg^{-1}	Initial soil	0.51±0.05						
	May	0.33±0.01^{b}	0.40±0.01^{a}	0.27±0.01^{d}	0.27±0.01^{d}	0.28±0.01^{d}	0.31±0.01^{c}	0.40±0.01^{a}
	August	0.32±0.01^{bc}	0.38±0.01^{a}	0.28±0.01^{c}	0.28±0.08^{c}	0.25±0.01^{c}	0.29±0.01^{ab}	0.40±0.01^{a}
	September	0.34±0.01^{ab}	0.42±0.01^{a}	0.18±0.12^{c}	0.24±0.01^{bc}	0.26±0.02^{bc}	0.30±0.02^{b}	0.41±0.02^{a}

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK); avl*-available element.

Table 12. Effect of different fertilizer treatments and element contents on the soil at different times of sampling during 2019

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
N total %	Initial soil	0.13±0.00						
	May	0.13±0.00	0.13±0.00	0.13±0.01	0.13±0.00	0.13±0.00	0.13±0.00	0.13±0.00
	August	0.12±0.00^{b}	0.13±0.00^{ab}	0.13±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{ab}	0.13±0.01^{ab}	0.13±0.00^{ab}
	September	0.13±0.01	0.12±0.00	0.13±0.00	0.13±0.00	0.13±0.00	0.12±0.00	0.12±0.00
N min kg ha^{-1}	Initial soil	38.21±1.78						
	May	60.46±2.52^{b}	60.46±2.52^{b}	60.46±2.52^{b}	60.46±2.52^{b}	60.46±2.52^{b}	60.46±2.52^{b}	60.46±2.52^{b}
	August	35.09±2.52^{c}	35.09±2.52^{c}	35.09±2.52^{c}	35.09±2.52^{c}	35.09±2.52^{c}	35.09±2.52^{c}	35.09±2.52^{c}
	September	35.91±1.73^{ab}	35.91±1.73^{ab}	35.91±1.73^{ab}	35.91±1.73^{ab}	35.91±1.73^{ab}	35.91±1.73^{ab}	35.91±1.73^{ab}
P %	Initial soil	0.09±0.00						
	May	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00
	August	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00
	September	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}
P – avl* mg 100 g^{-1}	Initial soil	14.44±1.33						
	September	11.00±0.84	11.00±0.84	11.00±0.84	11.00±0.84	11.00±0.84	11.00±0.84	11.00±0.84
K %	Initial soil	0.19±0.01						
	May	0.20±0.01^{ab}	0.20±0.01^{ab}	0.20±0.01^{ab}	0.20±0.01^{ab}	0.20±0.01^{ab}	0.20±0.01^{ab}	0.20±0.01^{ab}
	August	0.20±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02^{ab}
	September	0.22±0.01^{ab}	0.22±0.01^{ab}	0.22±0.01^{ab}	0.22±0.01^{ab}	0.22±0.01^{ab}	0.22±0.01^{ab}	0.22±0.01^{ab}
K – avl* mg 100 g^{-1}	Initial soil	18.50±1.07						
	September	16.10±0.82	16.10±0.82	16.10±0.82	16.10±0.82	16.10±0.82	16.10±0.82	16.10±0.82
Ca %	Initial soil	0.39±0.02						
	May	0.39±0.01	0.39±0.01	0.39±0.01	0.39±0.01	0.39±0.01	0.39±0.01	0.39±0.01
	August	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03
	September	0.36±0.03	0.36±0.03	0.36±0.03	0.36±0.03	0.36±0.03	0.36±0.03	0.36±0.03
Mg %	Initial soil	0.60±0.03						
	May	0.65±0.01^{b}	0.65±0.01^{b}	0.65±0.01^{b}	0.65±0.01^{b}	0.65±0.01^{b}	0.65±0.01^{b}	0.65±0.01^{b}
	August	0.66±0.02^{a}	0.66±0.02^{a}	0.66±0.02^{a}	0.66±0.02^{a}	0.66±0.02^{a}	0.66±0.02^{a}	0.66±0.02^{a}
	September	0.75±0.02	0.75±0.02	0.75±0.02	0.75±0.02	0.75±0.02	0.75±0.02	0.75±0.02

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Mn mg kg^{-1}	Initial soil	1.18±0.02						
	May	1.25±0.02	1.24±0.03	1.26±0.02	1.24±0.05	1.24±0.03	1.23±0.05	1.23±0.05
	August	1.20±0.04	1.26±0.24	1.33±0.19	1.24±0.06	1.29±0.06	1.27±0.05	1.25±0.05
	September	1.26±0.05	1.30±0.13	1.26±0.08	1.26±0.04	1.34±0.06	1.42±0.16	1.26±0.05
Fe g kg^{-1}	Initial soil	33.05±1.33						
	May	34.58±0.87	33.88±0.56	34.18±0.78	33.75±0.84	33.56±1.46	33.82±1.89	34.12±0.96
	August	35.66±1.03	36.84±4.45	35.78±1.04	35.53±0.66	36.25±1.40	36.14±0.62	35.19±0.88
	September	35.77±1.24	34.86±1.01	34.51±0.44	34.85±0.94	35.55±0.88	36.39±2.06	35.51±1.70
Zn mg kg^{-1}	Initial soil	96.52±1.20						
	May	75.32±1.54	71.42±3.84	72.28±1.21	74.36±0.63	71.81±3.32	74.54±3.09	72.711±3.95
	August	85.52±1.40	85.39±4.14	86.86±1.02	87.38±1.41	87.69±2.56	87.81±1.73	88.32±1.77
	September	66.32±1.53^{b}	66.14±1.09^{b}	66.12±1.13^{b}	65.55±1.02^{b}	67.04±1.22^{ab}	69.04±0.73^{a}	67.741.17^{ab}
Cu mg kg^{-1}	Initial soil	23.59±0.46						
	May	23.85±1.47	23.29±0.99	24.25±0.83	23.76±0.98	23.09±1.00	23.50±0.86	24.15±1.84
	August	31.40±3.96	26.74±1.05	28.62±1.02	29.43±0.96	29.07±1.22	29.65±1.14	29.73±2.08
	September	27.75±1.03	27.21±0.65	27.29±1.32	27.24±1.16	27.78±0.68	27.64±1.95	27.80±1.87
Ni mg kg^{-1}	Initial soil	31.80±0.45						
	May	30.70±1.31	29.45±1.17	28.60±0.36	30.07±0.42	29.15±1.01	29.90±1.31	29.97±2.16
	August	35.63±1.24	35.96±2.79	36.33±1.04	36.38±1.76	36.70±1.62	37.16±0.86	36.30±1.11
	September	30.29±1.73^{ab}	29.86±1.01^{b}	30.17±1.50^{ab}	30.69±2.51^{ab}	33.69±1.41^{ab}	34.04±2.17^{a}	31.2±1.25^{ab}
Pb mg kg^{-1}	Initial soil	18.25±0.29						
	May	24.85±2.53	25.25±1.93	21.45±2.06	26.30±2.96	24.11±5.03	23.78±2.12	25.35±3.12
	August	27.41±3.47	25.20±2.09	24.17±2.39	24.79±3.80	28.01±2.26	25.22±3.13	29.80±4.64
	September	28.13±2.83	29.91±2.84	28.54±2.20	29.26±4.90	29.30±3.66	33.73±3.48	29.23±2.44

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Cr mg kg^{-1}	Initial soil	39.69±1.14						
	May	42.73±0.60^{a}	41.44±0.15^{ab}	40.50±1.01^{b}	41.57±0.24^{ab}	40.85±1.59^{ab}	40.25±0.32^{b}	41.08±1.45^{ab}
	August	42.30±1.87	44.36±5.82	44.87±1.25	45.98±1.18	45.50±1.89	46.53±1.36	45.25±1.19
	September	40.03±0.57	39.10±1.15	38.10±0.93	38.43±1.16	39.11±0.57	38.14±1.62	38.68±1.10
Co mg kg^{-1}	Initial soil	19.86±0.56						
	May	18.80±1.28	19.38±0.55	19.60±0.90	18.91±0.64	18.79±0.49	19.03±1.11	18.87±1.05
	August	21.34±0.68	22.00±3.86	22.71±2.07	21.91±0.57	21.95±1.52	22.58±1.22	21.65±1.61
	September	17.66±4.00	16.87±2.07	17.00±0.84	16.89±1.43	18.37±2.46	17.15±2.23	14.50±2.38
Cd mg kg^{-1}	Initial soil	0.45±0.10						
	May	0.34±0.01^{d}	0.37±0.01^{ab}	0.34±0.02^{c}	0.35±0.01^{bc}	0.3±0.01^{cd}	0.34±0.01^{c}	0.38±0.01^{a}
	August	0.29±0.03^{b}	0.34±0.01^{a}	0.31±0.01^{ab}	0.33±0.01a	0.31±0.01^{ab}	0.32±0.01^{ab}	0.34±0.01^{a}
	September	0.37±0.01^{c}	0.40±0.01^{ab}	0.38±0.01^{c}	0.40±0.01^{ab}	0.40±0.02^{a}	0.40±0.01^{ab}	0.42±0.01^{a}

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK); avl*- available element.

4.2. PLANT CHARACTERISTICS

4.2.1. Plant Nutrient and Heavy Metal Dynamics Under Different Fertilizer Treatments (2018–2019)

Tables 13 (2018) and 14 (2019) present the effects of different fertilizer treatments on the nutrient status of maize plants across three sampling moments (V4, R5 and R6). The results provide an overview of how macronutrients, micronutrients, and trace (heavy metal) elements changed throughout the season and how these dynamics were influenced by the applied treatments.

In both years, total N concentrations were highest at the early vegetative stage (V4) and declined as the season progressed. Treatments combining mineral fertilizers with digestate, especially NPK+CAN, NPK+LFD, and NPK+SFD, produced the highest total N at the first sampling in both seasons, while the control and single digestate treatments typically had the lowest values. By the second and third samplings, differences between treatments became smaller as plant uptake and internal redistribution reduced overall N levels. A general downward trend in total N content toward the end of the growing season was consistent in both years.

Nitrate (NO_3^-) concentrations showed a similar but more pronounced early-season peak. In 2018, the highest NO_3^- levels were recorded in NPK+CAN and LFD+NPK in May, while treatments with digestate alone and the control showed lower values. By August, NO_3^- concentrations had declined sharply across all treatments. In 2019, early-season nitrate accumulation was again greatest in treatments combining mineral fertilizers with digestate (SFD+NPK, LFD+NPK, NPK+CAN), with the lowest values in the control, SFD, and LCM treatments. By August, nitrate levels dropped to similarly low concentrations across all treatments. These results indicate that mineral digestate combinations promote rapid nitrate availability and uptake early in the season, with strong depletion as the plants mature.

For the macronutrients P, K, Ca, and Mg, most concentrations were highest early in the season and gradually declined as plant biomass increased. In 2018, few significant differences in P were observed, except for a higher P content in SFD at the final sampling. In 2019, SFD again showed the highest P content at the first sampling, whereas NPK+CAN tended to show the lowest. Potassium showed clearer treatment responses, with LFD (and NPK+LFD) yielding the highest K concentrations early in the season in both years, while later samplings showed fewer differences as K levels decreased across all treatments. Calcium and magnesium remained comparatively stable, with only occasional significant differences (e.g., higher Ca in mineral-based treatments in mid-season 2018 and locally elevated Mg in NPK+LFD in late 2018). Overall, treatment effects on these macronutrients were modest and generally stage-dependent.

Micronutrients and trace elements displayed similar seasonal patterns, with high early-season concentrations followed by reductions linked mostly to dilution by plant growth rather than treatment effects. Iron was high in early samplings in both years, especially in 2019, and decreased sharply thereafter with minimal treatment differences. Manganese also declined strongly from the first to later samplings, again without significant treatment effects; this trend likely reflects the dilution effect caused by rapid biomass increase.

Zinc showed somewhat more variable treatment responses. In 2018, NPK+CAN produced the highest Zn concentrations early, while later peaks appeared in LCM and SFD. In 2019, Zn initially peaked under NPK+LFD, and by the final sampling the control had the highest Zn values, with NPK+CAN the lowest. These patterns suggest that fertilizer effects on Zn uptake were more pronounced early in the season and decreased with plant maturity.

Copper concentrations also followed a general decreasing trend, with small but notable treatment differences. In 2018, NPK+CAN and NPK+SFD supported higher Cu levels in the mid and late season, while the control and digestate-only treatments generally showed the lowest values. In 2019, LFD promoted higher early-season Cu uptake, whereas NPK+CAN and NPK+SFD tended to produce higher Cu later in the season.

Nickel, chromium, cobalt, cadmium, and lead showed limited treatment-driven variation. For Ni, concentrations remained largely unchanged across treatments in both years, with only slight shifts at the final sampling. Cr and Co displayed no significant treatment effects and followed a simple pattern of higher concentrations early in the season followed by stabilization. Cadmium showed small natural fluctuations but no significant treatment influence. Lead concentrations varied more between samplings and treatments, particularly in 2018, but overall decreased toward the end of the season, and differences in 2019 were mostly statistically insignificant due to high variability.

Overall, the results indicate that fertilizer treatments influenced nutrient accumulation mainly during early growth stages, with mineral–digestate combinations showing the strongest effects. As the season progressed, concentrations of most elements decreased due to plant development and nutrient dilution, reducing treatment-related differences by physiological maturity.

Table 13. Effect of different fertilizer treatments and element contents on maize at different times of sampling during 2018

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
N %	May	3.67±0.07^{b}	4.14±0.10^{a}	3.56±0.05^{b}	3.44±0.27^{b}	3.57±0.11^{b}	4.10±0.19^{a}	4.04±0.11^{a}
	August	0.71±0.06^{c}	0.94±0.01^{a}	0.85±0.09^{ab}	0.76±0.04^{bc}	0.77±0.06^{bc}	0.86±0.04^{ab}	0.94±0.08^{a}
	September	0.80±0.04^{c}	0.96±0.02^{a}	0.85±0.04^{bc}	0.86±0.02^{bc}	0.92±0.02^{ab}	0.91±0.03^{ab}	0.91±0.02^{ab}
NO^{3-} mg kg^{-1}	May	1047±181^{abc}	1285±49^{a}	819±90^{c}	758±179^{c}	941±110^{bc}	1183±125^{ab}	1268±132^{a}
	August	369±133^{c}	821±96^{a}	542±61^{b}	285±41^{c}	312±34^{c}	382±33^{c}	408±76^{bc}
P %	May	0.28±0.02	0.30±0.01	0.26±0.01	0.27±0.01	0.26±0.02	0.29±0.01	0.29±0.01
	August	0.16±0.02	0.15±0.01	0.17±0.01	0.16±0.01	0.15±0.02	0.13±0.02	0.15±0.01
	September	0.17±0.02^{ab}	0.14±0.01^{b}	0.17±0.02^{ab}	0.19±0.02^{a}	0.16±0.02^{ab}	0.17±0.01^{ab}	0.16±0.00^{ab}
K %	May	3.55±0.28^{b}	3.37±0.10^{b}	3.29±0.08^{b}	3.41±0.29^{b}	3.23±0.04^{b}	3.33±0.12^{b}	4.06±0.05^{a}
	August	0.81±0.07	0.89±0.05	0.88±0.07	0.86±0.07	0.88±0.04	0.92±0.10	0.82±0.04
	September	0.63±0.03^{ab}	0.57±0.03^{ab}	0.60±0.07^{ab}	0.64±0.05^{a}	0.62±0.04^{ab}	0.60±0.01^{a}	0.54±0.04^{b}
Ca %	May	0.68±0.03	0.69±0.02	0.69±0.03	0.68±0.02	0.70±0.03	0.71±0.01	0.72±0.03
	August	0.18±0.00^{b}	0.23±0.03^{a}	0.20±0.02^{ab}	0.19±0.02^{ab}	0.19±0.01^{ab}	0.23±0.02^{a}	0.21±0.03^{ab}
	September	0.16±0.03	0.15±0.01	0.17±0.02	0.17±0.03	0.18±0.03	0.17±0.04	0.19±0.00
Mg %	May	0.20±0.00^{b}	0.22±0.01^{ab}	0.23±0.01^{ab}	0.22±0.01^{ab}	0.23±0.02^{a}	0.21±0.02^{ab}	0.22±0.01^{ab}
	August	0.12±0.00	0.14±0.02	0.13±0.00	0.13±0.01	0.12±0.01	0.13±0.01	0.13±0.02
	September	0.13±0.01^{b}	0.11±0.00^{b}	0.13±0.01^{b}	0.14±0.01^{b}	0.22±0.10^{ab}	0.20±0.10^{ab}	0.27±0.02^{a}
Fe mg kg^{-1}	May	395.7±73.4	330.2±59.0	356.2±144.4	343.2±44.5	386.5±48.1	265.2±5.8	312.5±36.7
	August	76.5±2.4^{bc}	69.8±4.6^{c}	85.6±5.2^{abc}	87.3±11.9^{ab}	95.4±1.9^{a}	86.1±8.2^{abc}	83.9±11.7^{abc}
	September	127.2±18.1	108.0±21.8	118.1±21.2	118.8±36.0	101.7±9.3	134.5±30.8	103.2±8.3
Mn mg kg^{-1}	May	111.7±18.1	160.5±24.7	121.8±34.7	117.1±3.3	141.7±50.6	172.4±27.9	164.1±34.0
	August	29.6±1.6	36.3±13.1	29.9±2.6	29.9±5.4	31.5±0.8	36.2±9.2	33.9±1.5
	September	27.1±5.5	24.9±2.8	25.3±4.3	29.2±5.4	25.8±1.2	29.4±2.0	26.0±2.2

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Zn mg kg^{-1}	May	37.26±2.99^{bc}	48.1±2.78^{a}	40.62±2.75^{abc}	35.65±7.07^{c}	42.15±3.43^{abc}	44.86±3.02^{ab}	44.41±1.98^{ab}
	August	18.06±0.73^{ab}	14.18±3.96^{ab}	19.69±3.40^{a}	17.37±1.82^{ab}	17.46±1.95^{ab}	13.96±2.16^{ab}	12.93±3.50^{b}
	September	19.92±1.08^{ab}	14.98±0.97^{c}	20.16±1.93^{a}	20.24±1.31^{a}	18.38±2.63^{abc}	17.89±0.85^{abc}	16.58±0.98^{bc}
Cu mg kg^{-1}	May	8.64±0.41	8.47±0.34	8.78±0.43	10.33±0.44	8.81±0.92	8.32±0.83	8.48±0.43
	August	0.95±0.16^{b}	2.16±0.72^{a}	1.51±0.53^{ab}	1.15±0.25^{b}	1.03±0.14^{b}	1.36±0.52^{ab}	1.30±0.31^{ab}
	September	1.30±0.21^{b}	2.03±0.72^{ab}	1.72±0.29^{ab}	1.97±0.35^{ab}	2.01±0.52^{ab}	2.55±0.38^{a}	2.05±0.44^{ab}
Ni mg kg^{-1}	May	6.79±1.42	8.35±1.70	8.21±4.31	7.60±0.90	7.59±3.44	6.16±3.38	6.04±4.07
	August	2.40±0.95^{a}	0.49±0.22^{b}	1.37±1.11^{ab}	0.38±0.38^{b}	1.79±0.46^{ab}	0.59±0.37^{b}	1.66±0.62^{ab}
	September	1.29±0.43^{b}	2.03±0.27^{ab}	2.18±0.44^{ab}	2.17±0.62^{ab}	2.38±0.10^{a}	2.00±0.46^{ab}	2.92±0.58^{a}
Pb mg kg^{-1}	May	3.67±1.71^{ab}	6.17±0.93^{a}	6.20±0.58^{a}	2.25±1.92^{b}	3.25±1.52^{ab}	2.85±1.74^{ab}	2.10±1.37^{b}
	August	3.97±1.17^{ab}	1.38±0.65^{c}	1.91±0.49^{bc}	2.56±1.62^{abc}	4.65±0.76^{a}	2.59±0.87^{abc}	1.64±0.18^{c}
	September	1.93±0.75^{b}	1.89±0.69b	2.27±1.26^{b}	2.43±0.96^{ab}	3.86±1.60^{ab}	4.67±0.64^{a}	3.68±0.83^{ab}
Cr mg kg^{-1}	May	11.43±1.17	15.42±5.43	10.23±2.69	14.25±2.85	11.68±1.62	10.40±2.77	11.37±1.91
	August	4.67±1.32	3.61±0.96	4.73±0.47	4.50±0.30	5.73±1.34	4.23±0.90	3.69±0.87
	September	4.31±0.74	3.83±0.23	3.46±0.77	2.91±0.65	3.43±0.76	3.39±0.35	3.41±0.87
Co mg kg^{-1}	May	1.51±0.57	1.27±0.79	2.09±1.00	0.85±0.47	2.01±0.45	1.22±1.12	0.87±0.32
	August	0.61±0.28	0.44±0.33	0.23±0.29	0.77±0.61	1.16±1.22	0.86±0.52	0.43±0.18
	September	0.35±0.26	0.46±0.06	0.59±0.23	0.47±0.24	0.58±0.45	0.52±0.23	0.55±0.16
Cd mg kg^{-1}	May	0.47±0.20	0.70±0.34	0.54±0.26	0.90±0.48	0.55±0.23	0.64±0.36	0.93±0.33
	August	0.24±0.14	0.28±0.20	0.30±0.22	0.26±0.08	0.34±0.18	0.28±0.21	0.28±0.11
	September	0.44±0.16	0.41±0.12	0.45±0.10	0.43±0.06	0.42±0.12	0.48±0.10	0.58±0.06

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

Table 14. Effect of different fertilizer treatments and element contents on maize at different times of sampling during 2019

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
N %	June	3.40±0.15[bc]	3.70±0.04[a]	3.56±0.09[abc]	3.36±0.13[c]	3.76±0.04[a]	3.64±0.18[ab]	3.78±0.03[a]
	August	0.64±0.04[c]	1.08±0.09[a]	0.72±0.11[bc]	0.68±0.01[bc]	0.77±0.07[bc]	0.81±0.08[b]	0.83±0.04[b]
	October	0.66±0.04[c]	0.88±0.03[a]	0.77±0.04[b]	0.67±0.03[c]	0.80±0.02[b]	0.83±0.02[ab]	0.81±0.01[ab]
NO^{3-} mg kg^{-1}	June	644±133[bc]	1303±117[a]	735±179[bc]	577±82[c]	1010±286[ab]	1413±155[a]	1338±261[a]
	August	159±42	174±28	160±20	160±20	172±30	181±26	176±46
P %	June	0.34±0.05[ab]	0.25±0.02[b]	0.34±0.03[ab]	0.35±0.05[a]	0.33±0.03[ab]	0.28±0.03[ab]	0.31±0.03[ab]
	August	0.12±0.03	0.1±0.01	0.11±0.01	0.13±0.01	0.13±0.03	0.14±0.02	0.14±0.01
	October	0.16±0.02	0.15±0.01	0.16±0.01	0.16±0.01	0.16±0.01	0.16±0.02	0.16±0.01
K %	June	4.02±0.16[b]	4.55±0.16[ab]	4.27±0.17[ab]	4.13±0.20[ab]	4.62±0.38[a]	4.52±0.01[ab]	4.57±0.32[ab]
	August	0.89±0.04	0.91±0.04	0.91±0.07	0.85±0.05	0.82±0.05	0.82±0.04	0.87±0.12
	October	0.62±0.03	0.54±0.03	0.64±0.00	0.67±0.01	0.67±0.03	0.68±0.04	0.65±0.03
Ca %	June	0.66±0.03	0.50±0.33	0.60±0.05	0.65±0.01	0.61±0.02	0.44±0.30	0.62±0.02
	August	0.24±0.00[ab]	0.27±0.03[a]	0.25±0.01[ab]	0.22±0.02[b]	0.22±0.02[b]	0.24±0.02[ab]	0.25±0.02[ab]
	October	0.13±0.03	0.16±0.03	0.14±0.00	0.13±0.01	0.16±0.03	0.14±0.04	0.15±0.03
Mg %	June	0.24±0.01	0.16±0.11	0.23±0.02	0.25±0.02	0.23±0.01	0.16±0.11	0.23±0.02
	August	0.12±0.01	0.13±0.01	0.12±0.01	0.11±0.00	0.12±0.01	0.12±0.00	0.12±0.01
	October	0.11±0.01	0.11±0.01	0.11±0.00	0.10±0.00	0.11±0.00	0.10±0.01	0.11±0.01
Fe mg kg^{-1}	June	951.2±306.2	752.2±219.2	803.5±221.4	948.3±195.3	810.3±57.3	601.2±28.1	736.6±63.3
	August	182.0±29.9[a]	102.4±18.6[c]	139.5±10.3[abc]	158.6±28.9[ab]	151.8±27.3[abc]	123.9±11.8[bc]	110.2±27.3[bc]
	October	205.8±50.1	133.1±35.5	145.8±40.3	155.9±27.9	143.4±36.8	137.4±24.7	152.2±32.5
Mn mg kg^{-1}	June	80.66±19.05	83.99±15.91	65.12±11.14	80.43±18.96	73.27±10.61	77.92±8.98	71.44±7.45
	August	38.16±2.56	42.31±2.66	37.97±2.77	34.80±4.11	35.43±5.82	38.67±4.86	35.47±3.91
	October	25.50±5.13	24.01±4.20	22.93±2.93	24.03±0.18	28.61±4.80	28.22±7.37	25.11±3.13

Element	Month/ Sampling moment	Treatment						
		C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Zn mg kg^{-1}	June	30.64±2.48^{ab}	29.19±1.36^{abc}	28.07±1.98^{abc}	25.49±0.26^{c}	30.27±1.88^{ab}	27.16±1.17^{bc}	31.46±0.83^{a}
	August	14.02±1.78	14.22±1.97	14.62±1.57	15.39±2.03	16.41±1.94	15.48±1.90	15.01±1.58
	October	16.62±1.72^{a}	13.24±0.64^{b}	15.55±1.05^{ab}	14.75±0.50^{ab}	15.16±2.31^{ab}	14.03±1.96^{ab}	14.04±1.09^{ab}
Cu mg kg^{-1}	June	12.46±0.69^{ab}	12.04±0.94^{ab}	12.42±0.12^{ab}	12.93±1.51^{ab}	13.20±0.82^{a}	10.71±0.68^{b}	12.83±1.20^{ab}
	August	2.38±0.32^{b}	4.20±0.21^{a}	2.97±0.36^{ab}	2.56±0.38^{b}	3.17±0.55^{ab}	3.39±0.43^{ab}	3.51±1.11^{ab}
	October	2.03±0.37^{b}	2.77±0.51^{ab}	2.35±0.16^{ab}	2.20±0.24^{b}	3.00±0.41^{a}	3.05±037^{a}	2.73±0.21^{ab}
Ni mg kg^{-1}	June	3.88±2.18	3.14±3.27	2.89±1.13	4.54±1.81	2.16±2.41	3.68±2.20	3.03±2.50
	August	4.47±2.09	3.10±0.55	3.47±0.96	3.92±0.49	4.37±1.02	3.96±0.85	3.21±1.26
	October	1.61±0.25^{b}	2.41±0.69^{ab}	2.18±0.39^{ab}	2.42±0.58^{ab}	2.54±0.14^{ab}	2.21±0.48^{ab}	3.16±0.56^{a}
Pb mg kg^{-1}	June	3.08±0.87	4.33±1.99	4.52±0.88	5.36±2.53	8.34±0.96	5.16±0.42	3.69±1.40
	August	3.35±3.61	5.46±3.41	6.14±2.59	1.85±1.15	2.58±1.64	6.24±1.65	3.92±2.75
	October	2.12±0.66^{b}	2.04±0.62^{b}	2.43±1.21^{b}	2.54±0.85^{b}	3.96±1.56^{ab}	4.82±0.72^{a}	3.83±0.79^{ab}
Cr mg kg^{-1}	June	14.45±1.87	12.02±2.11	11.84±1.02	15.04±3.32	11.88±3.07	11.15±0.30	11.45±1.23
	August	5.28±1.13	3.79±0.92	4.31±0.50	4.78±0.46	3.95±0.83	4.40±0.38	4.15±0.67
	October	4.48±0.76	3.93±0.16	3.63±0.72	3.05±0.61	3.59±0.76	3.49±0.42	3.58±0.90
Co mg kg^{-1}	June	1.15±0.46	0.78±0.26	1.35±0.74	0.60±0.23	0.63±0.71	0.32±0.34	1.09±0.54
	August	0.79±0.71	0.70±0.19	0.67±0.52	0.76±0.42	0.51±0.24	0.69±0.38	0.78±0.45
	October	0.48±0.28	0.56±0.06	0.69±0.23	0.55±0.21	0.66±0.34	0.63±0.23	0.66±0.14
Cd mg kg^{-1}	June	0.38±0.24	0.43±0.20	0.22±0.15	0.25±0.25	0.39±0.22	0.38±0.23	0.24±0.15
	August	0.22±0.21	0.44±0.06	0.21±0.06	0.43±0.24	0.31±0.08	0.42±0.11	0.20±0.10
	October	0.56±0.07	0.48±0.12	0.57±0.13	0.54±0.08	0.52±0.15	0.59±0.09	0.67±0.03

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3. NUTRIENT UPTAKE, ANR, NFRV, NITRATES AND YIELD

4.3.1. Maize nutrient uptake

ANOVA was conducted to evaluate how different years (2018 and 2019) and fertilizer treatments affected the uptake of various macro- and micronutrients by maize, including N, P, K, Ca, Mg, Fe, Zn, Mn and Cu.

The results showed a statistically significant interaction between year and fertilizer treatment for the uptake of N ($p=0.0090$), K ($p=0.0152$) and Cu ($p=0.0263$). Further analysis of the main effects showed that the average uptake of N, P, Zn and Cu (measured in kg ha^{-1}) was higher in 2018, while the highest uptake of Ca, Mg, Fe and Mn occurred in 2019. Interestingly, no significant difference in K uptake was found between the two years.

Among the treatments, the highest N uptake was observed in the NPK+CAN treatment (271.9 kg ha^{-1}), while treatments combining NPK with either solid or liquid digestate (SFD or LFD) also resulted in significantly higher N uptake compared to LFD, SFD, LCM and C treatment. The significantly lowest N uptake was at control treatment (153,1 kg ha^{-1}). Phosphorus uptake was lowest in the LFD and C, while K uptake was lowest in the LFD, LCM and C treatment. However, no significant differences in P and K uptake were observed between the other treatments. Calcium uptake peaked in the NPK+CAN and LFD+NPK treatments. Magnesium and Cu uptake was also highest in the NPK+CAN treatment, while the lowest values were recorded in the C treatment. The uptake of Mn was generally highest in the NPK+CAN and SFD+NPK treatments. On the other hand, the uptake of Fe and Zn did not differ significantly between the two years (Table 15).

Table 15. Total average maize nutrient uptake for the seven different fertilization treatments (2018-2019)

Parameters	C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Total N (kg ha^{-1})	153.1^{d}	271.9^{a}	201.7^{c}	194.6^{c}	209.7^{c}	231.8^{b}	249.3^{b}
Total P (kg ha^{-1})	56.2^{b}	70.7^{a}	67.3^{a}	67.3^{a}	65.0^{ab}	68.0^{a}	70.6^{a}
Total K (kg ha^{-1})	141.6^{b}	181.9^{a}	165.7^{ab}	179.7^{a}	167.5^{ab}	181.6^{a}	189.6^{a}
Total Ca (kg ha^{-1})	27.4^{b}	43.5^{a}	35.2^{ab}	34.2^{ab}	35.5^{ab}	37.5^{ab}	41.3^{a}
Total Mg (kg ha^{-1})	21.0^{c}	29.3^{a}	25.6^{b}	25.6^{b}	25.1^{b}	26.3^{ab}	28.4^{ab}
Total Fe (kg ha^{-1})*	3.5	3.4	3.3	3.6	3.1	3.7	3.7
Total Zn (kg ha^{-1})**	0.36	0.40	0.41	0.39	0.38	0.41	0.40
Total Mn (kg ha^{-1})	0.57^{b}	0.77^{a}	0.62^{ab}	0.66^{ab}	0.68^{ab}	0.78^{a}	0.76^{ab}
Total Cu (kg ha^{-1})	0.07^{d}	0.12^{a}	0.09^{bc}	0.08^{cd}	0.09^{bc}	0.10^{ab}	0.10^{ab}

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3.2. ANR and NFRV

As shown in Table 16, a statistically significant interaction was observed between the effects of year and fertilizer treatment ($p < 0.0012$). Further analysis of the simple main effects revealed that Apparent Nitrogen Recovery (ANR) was significantly higher in 2019 than in 2018 ($p < 0.0001$). In 2018, the NPK+CAN treatment and LFD+NPK showed significantly higher ANR values than the other treatments. Similarly, in 2019, the NPK+CAN treatment, SFD+NPK and LFD+NPK showed a significantly higher ANR compared to the other fertilization treatments.

The Nitrogen Fertilizer Replacement Value (NFRV) is a widely used indicator for evaluating the effectiveness of organic fertilizers as a substitute for mineral N. It reflects the proportion of mineral fertilizer N that can be replaced by N from organic sources (Sigurnjak et al., 2017) and serves as a useful guide for determining appropriate application rates of organic fertilizers. In both years of the study, the highest NFRV values were recorded in the NPK+CAN and LFD+NPK treatments, further confirming the superior performance of these fertilization strategies in improving N availability and uptake.

Table 16. Apparent nitrogen recovery (ANR) and nitrogen fertilizer replacement value (NFRV) ta-ble for the seven different fertilization treatments

Treatment	ANR		NFRV %	
	2018	2019	2018	2019
C	-	-	-	-
NPK+CAN	0.77±0.08	0.93±0.08	100±0	100±0
LCM	0.32±0.04	0.38±0.09	41±10	41±5
SFD	0.30±0.04	0.29±0.06	39±7	31±6
LFD	0.36±0.01	0.45±0.06	47±7	48±2
SFD+NPK	0.41±0.06	0.72±0.09	53±10	77±8
LFD+NPK	0.60±0.07	0.77±0.09	78±10	83±9

Note. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3.3. Soil NO_3^-−N and NH_4^+-N dynamics over two consecutive years

A one-way ANOVA was conducted to evaluate nitrate (NO_3^-−N) residue levels in soil across seven fertilizer treatments on multiple sampling dates in 2018 and 2019. The analysis revealed statistically significant differences in NO_3^-−N concentrations at several sampling moments, particularly during the early growing season in May and June of both years. On 21 May, 2018, the highest NO_3^-−N residue was measured in the LFD+NPK treatment (149.2 kg ha^{-1}), which was significantly higher than in all other treatments. Elevated NO_3^-−N levels were also observed in the NPK+CAN (109.6 kg ha^{-1}), SFD+NPK (94.3 kg ha^{-1}) and LCM (79.8 kg ha^{-1}) treatments, with clear treatment effects evident from the non-overlapping error bars. In contrast, NO_3^-−N concentrations measured at the end of August and in September 2018 were significantly lower (between 15 and 45 kg ha^{-1}) and no statistically significant differences were observed between treatments, as indicated by the overlapping standard deviations.

In 2019, the highest NO_3^-−N residues were measured on 6 June in the LFD (93.6 kg ha^{-1}), SFD+NPK (79.9 kg ha^{-1}) and LFD+NPK (65.9 kg ha^{-1}) treatments, while the C and SFD treatments had significantly lower values (48.1 and 53.6 kg ha^{-1}, respectively). A particularly pronounced treatment effect was observed on 22 August, 2019, when NPK+CAN (113.7 kg ha^{-1}) and LFD+NPK (107.4 kg ha^{-1}) significantly exceeded all other treatments, especially the C, which had minimal residues (3.1 kg ha^{-1}). Post harvest measurements in October of both years showed consistently low NO_3^-−N levels (generally below 20 kg ha^{-1}), with no significant differences between treatments due to narrow ranges and overlapping margins of error.

Figure 20 shows the NO_3-−N and NH4+–N dynamics in the 0–90 cm soil layer across seven fertilizer treatments during the 2018 and 2019 growing seasons. The highest NO_3-−N concentrations were recorded early in the season, particularly in May and June, with the LFD+NPK, NPK+CAN and SFD+NPK treatments showing markedly elevated values compared with the others, while C, SFD, and LFD maintained consistently low residual levels. Toward the end of each season, NO_3-−N concentrations converged across treatments, indicating limited post-harvest accumulation. NH4+–N showed a similar temporal pattern, with pronounced early-season peaks under mineral fertilizer treatments and much lower, more stable values in the control and purely organic treatments. The statistical analysis supports these observed dynamics; multivariate tests revealed highly significant effects of time for both NO_3-−N and NH4+–N ($p<0.001$; partial $\eta^2 > 0.98$), indicating strong seasonal fluctuations in mineral N availability. Mauchly’s test indicated that sphericity was violated for both datasets, and Greenhouse–Geisser corrected repeated measures ANOVA confirmed that time had a very large effect on mineral N levels ($p<0.001$; partial η^2=0.86–0.93). Furthermore, significant time × treatment interactions ($p<0.001$; partial η^2=0.61–0.77) demonstrated that fertilizer treatments differed not only in magnitude but also in the timing and shape of their N response curves. Overall, mineral N residues (NO_3-−N and NH4+–N) were generally higher in 2018 than in 2019, likely reflecting year to year differences in temperature, precipitation, and mineralization rates influencing N transformation processes.

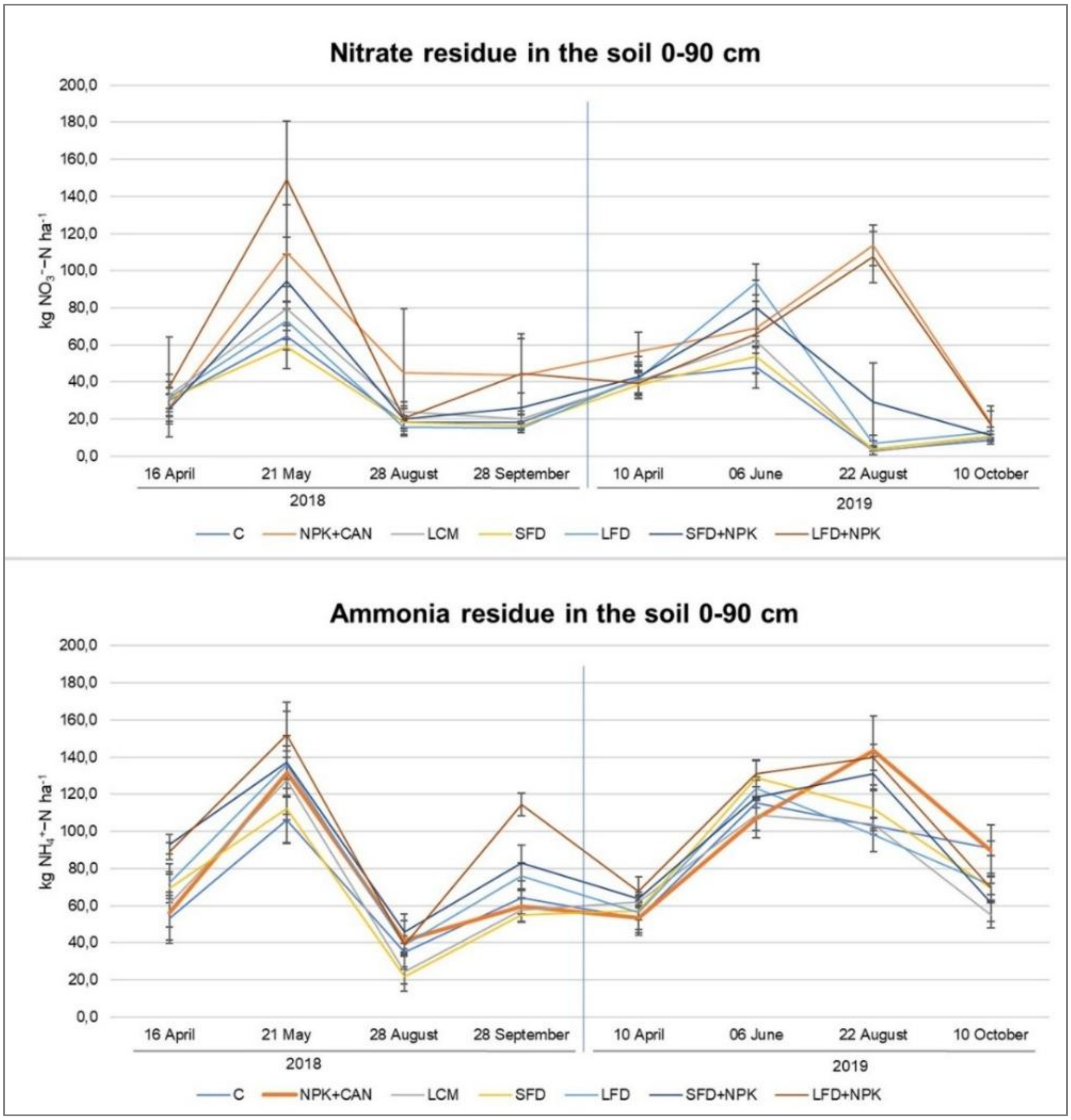

Figure 20. Dynamics of soil nitrate ($NO3^-$) and ammonium ($NH4^+$) residues (0–90 cm)

Note. 1-unfertilized control (C); 2-mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3.4. Nitrate residue in the soil

In a comparison of the two years, nitrate residues (kg ha-1) in the last sampling (end of the experiment) were generally higher in 2018 than in 2019. In 2018, the NPK+CAN treatment and the combined applications of SFD+NPK and LFD+NPK resulted in significantly higher nitrate levels in the soil compared to the other treatments. In 2019, however, these differences were not statistically significant as nitrate residues were more consistent between treatments (Figure 21).

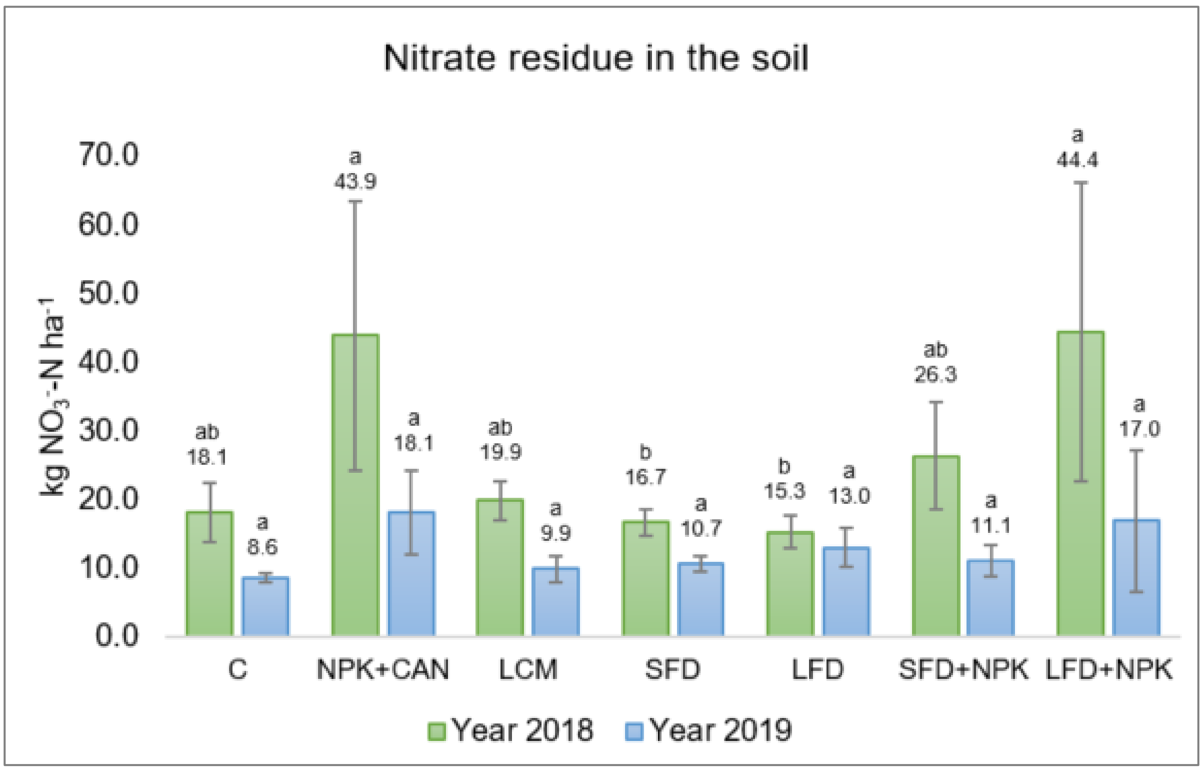

Figure 20. Post-harvest soil nitrate (0–90 cm) residue during 2018-2019

Note. Letters (a and b) indicate significant differences between groups for 2018 ($p<0.003$) and for 2019 ($p<0.79$). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

In both growing seasons, total soil mineral nitrogen (TSMN) did not differ significantly among treatments before the experiment was established and fertilization was applied. However, fertilization and crop development caused significant treatment effects, with TSMN generally increasing from pre-sowing to the R5 stage and then declining sharply at R6 (Figure 22).

In 2018, the highest TSMN concentrations were observed during the V4 vegetative stage in the NPK+CAN and LFD+NPK treatments, with values significantly higher than in the other treatments. Up to the R5 stage, the statistical differences between treatments persisted ($p < 0.05$), with the C treatment having the lowest TSMN content. At the R6 stage, the highest TSMN content was found in plots treated with NPK+CAN, SFD+NPK and LFD+NPK mixtures.

In 2019, the LFD, SFD+NPK and LFD+NPK treatments had the highest TSMN levels at the V4 stage. In R5, NPK+CAN and LFD+NPK again had the highest TSMN levels. In R6, the highest TSMN values were observed in the NPK+CAN, SFD, SFD+NPK and C treatments.

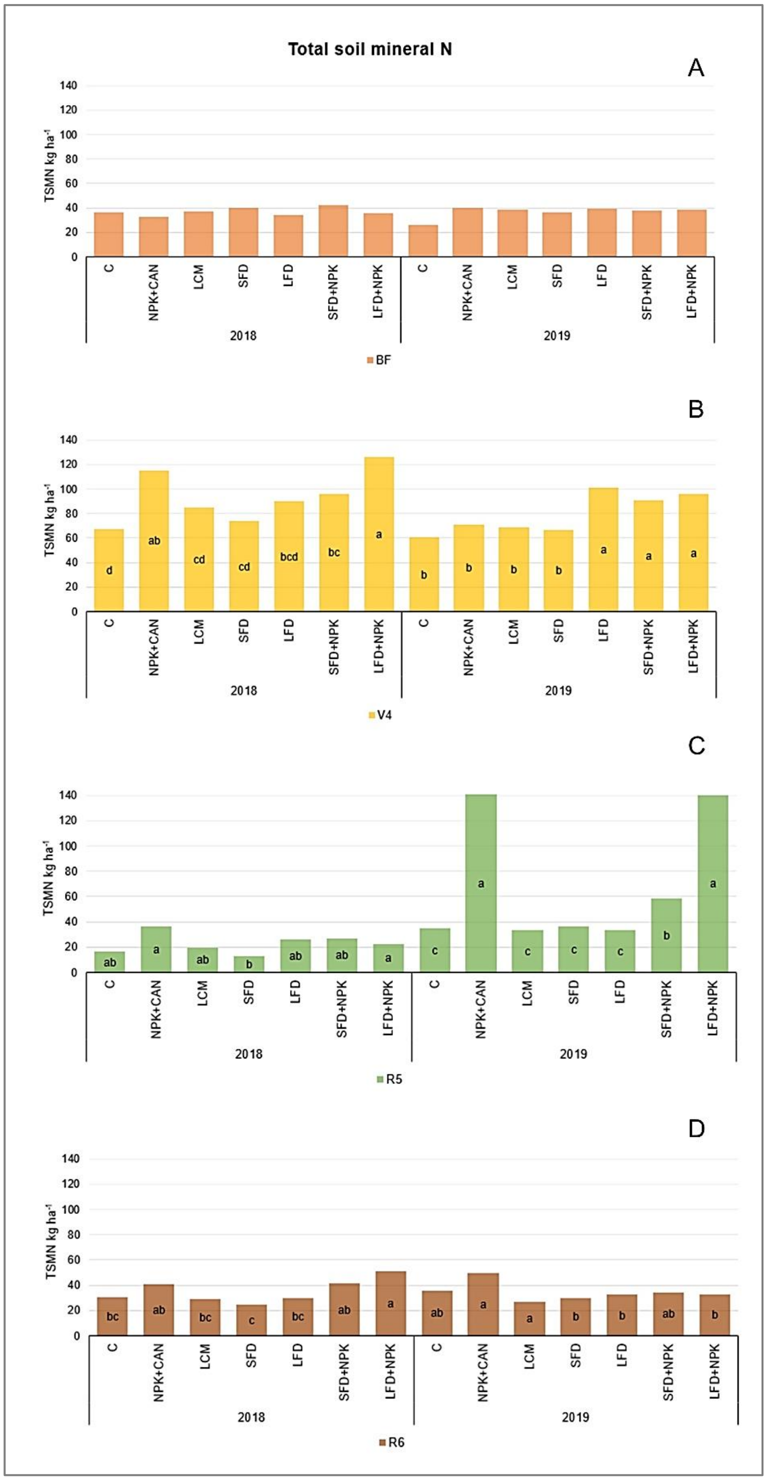

Figure 21. Total soil mineral nitrogen (TSMN) (0-30 cm) kg NO_3^- ha^{-1} before fertilization (A) and after fertilization (B, C and D) for two consecutive years during maize growth

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK). BF-before fertilization; V4-vegetative stage four fully developed leaves; R5-reproductive dent stage; R6-phisiological maturity.

4.3.5. Maize yield (fresh and dry biomass yield)

A statistically significant interaction was observed between year (2018 and 2019) and vegetation stage (V4, R5 and R6) as well as between fertilizer treatment and vegetation stage for both fresh and dry biomass yields (Table 17). A simple analysis of the main effects showed that higher yields of fresh and dry biomass were achieved in 2019 (38.45 t ha^{-1} FW and 15.7 t ha^{-1} DW) than in 2018 (30.9 t ha^{-1} FW and 14.4 t ha^{-1} DW). In both years, the highest average yields of fresh and dry biomass were recorded in the NPK+CAN treatments (39.6 t ha^{-1} FW and 17.4 t ha^{-1} DW) and the combination of LFD+NPK (37.5 t ha^{-1} FW and 16.5 t ha^{-1} DW), while the lowest yields were consistently observed in the C treatment (29.6 t ha^{-1} FW and 12.7 t ha^{-1} DW). In both years, fresh and dry biomass yields increased significantly from the V4 vegetative stage to the R6 reproductive stage, with particularly high yields recorded at the R5 and R6 stages in 2019 compared to 2018. No significant differences were found between years in the yields of fresh and dry biomass during the early V4 stage (Appendix, Table 3, 4 and 5).

Fertilizer treatments also had no significant effect on FW and DW yields at the V4 stage. However, as the plants developed, the differences between treatments became more pronounced, with the NPK+CAN and LFD+NPK treatments producing the highest FW and DW yields at the R5 and R6 stages, while the C treatment consistently produced the lowest yields (Šatvar Vrbančić et al., 2024).

Table 17. ANOVA table with *P* values for fresh and dry weight of above ground biomass yield during three vegetative stages (V4, R5 and R6) of maize growth

Source	**Df**	**FW yield t ha^{-1}**	**DW yield t ha^{-1}**
		***P* value**	
Year	1	<.0001	<.0001
Treatment	6	<.0001	<.0001
year*treatment	6	0.0714	0.1810
vegetative stage	2	<.0001	<.0001
year*vegetative stage	2	<.0001	<.0001
treatment*vegetative stage	12	<.0001	<.0001
year*treatment*vegetative stage	12	0.5710	0.7514

Note. FW-fresh weight of above-ground biomass yield; DW-dry weight of above ground biomass yield

The highest dry grain yield (Figure 23) was achieved in 2018 (10.9 t ha^{-1}) and thus significantly exceeded the yield of 2019 (9.6 t ha^{-1}) ($p<0.0001$). It appears that P and K from NPK fertilizers in combination with LFD had a synergistic effect and contributed to the higher yields. Statistically, similar results were also observed in the SFD+NPK treatment. Overall, the NPK+CAN treatment (12.1 t ha^{-1}) the combination of LFD+NPK (11.9 t ha^{-1}) and SFD+NPK (11 t ha^{-1}) produced significantly higher yields compared to the other treatments (Figure 66). In 2018, the highest dry grain yields were recorded for the NPK+CAN (13.1 t ha^{-1}), SFD+NPK (11.2 t ha^{-1}) and LFD+NPK (12.2 t ha^{-1}) treatments.

A similar trend was observed in 2019, with the NPK+CAN (11.1 t ha^{-1}), SFD+NPK (10.8 t ha^{-1}) and LFD+NPK (11.5 t ha^{-1}) treatments achieving the best results. In contrast, the lowest dry grain yield in both years was consistently recorded in the C treatment (Šatvar Vrbančić et al., 2024).

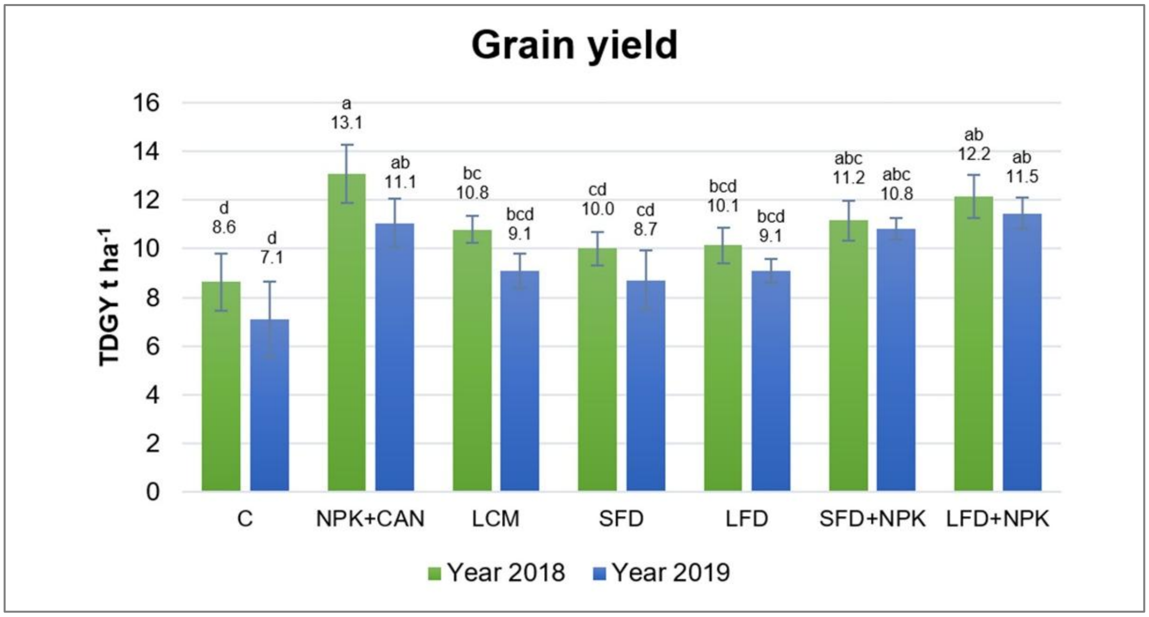

Figure 22. Total dry grain yield (TDGY) t ha^{-1} during two consecutive years

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3.6. Effect of treatment on mass balances

Table 18 and Figure 23 show the overall mass balances (kg ha^{-1}), calculated as fertilizer input minus plant uptake for the entire experimental period. Different letters within rows indicate statistically significant differences among treatments.

Nitrogen balance was negative in all treatments, indicating that crop removal exceeded fertilizer input. The largest deficit was observed in the C treatment, followed by NPK+CAN and LFD+NPK, while the smallest deficit occurred in the SFD and LCM treatments.

Phosphorus balance varied among treatments, with both negative and positive values observed. The highest positive balances were recorded in the NPK+CAN, SFD+NPK and LFD+NPK treatments, while the C and LFD treatments showed negative balances. Intermediate values were observed in the remaining treatments.

Potassium balance was negative in all treatments. The largest deficit occurred in the C and SFD treatments, while smaller deficits were observed in the LCM and LFD+NPK treatments.

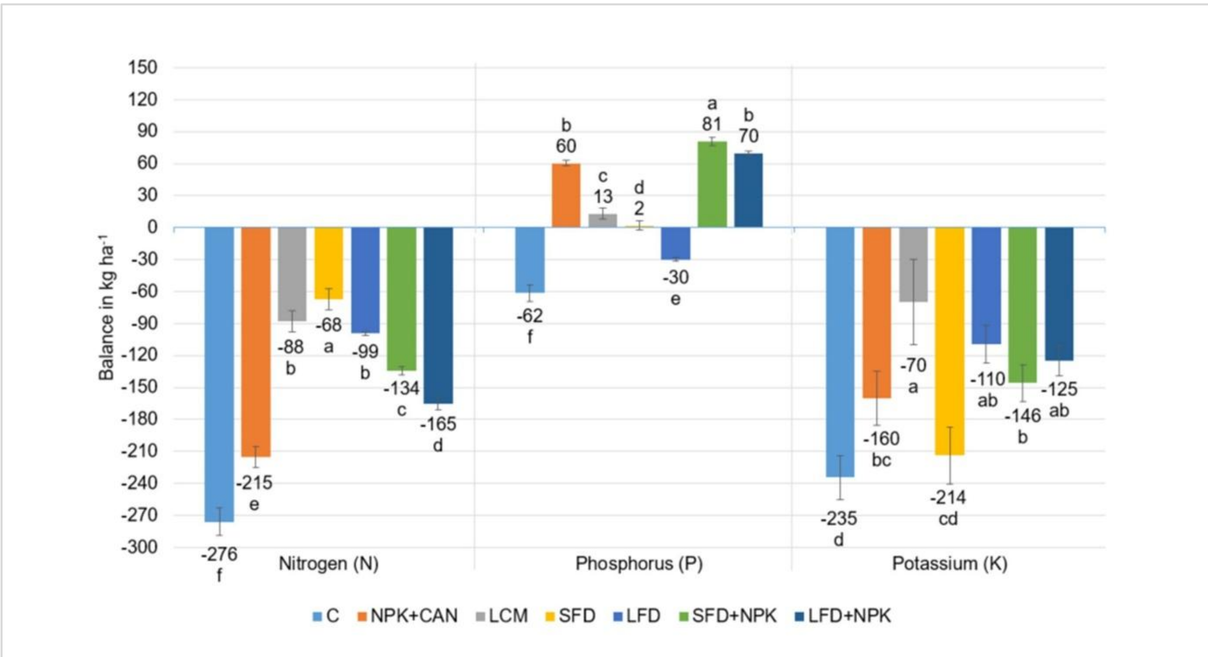

Figure 23. Nitrogen (N), phosphorus (P), and potassium (K) balances under different fertilizer treatments (sum for 2018–2019)

Furthermore, Mg balance varied among treatments with a slight positive balance observed in the LCM treatment. Other treatments exhibited negative balances, with the largest deficit recorded in the LFD+NPK treatment.

Calcium balance differed considerably among treatments. A strongly positive balance was observed in the LCM treatment, while a moderate positive balance occurred in the SFD treatment. All other treatments showed negative balances, with the largest deficits observed in the Control and LFD+NPK treatments.

Changes in Fe concentration were small across treatments, ranging from negative values in most treatments (Control, NPK+CAN, SFD+NPK, and LFD+NPK) to a slight increase in the LCM treatment, which showed the highest value.

Manganese changes were also limited in magnitude, with slight increases observed in the LCM and SFD treatments, while most other treatments showed small decreases.

Zinc concentrations showed minor variation, with the LCM treatment exhibiting the highest increase, while most other treatments showed small decreases.

Copper changes followed a similar pattern, with the largest increase observed in the LCM treatment and smaller increases in the SFD and LFD treatments, while the C and NPK+CAN treatments showed slight decreases.

Nickel concentrations decreased in all treatments, with the largest reduction observed in the LFD+NPK treatment, while the C and LCM treatments showed comparatively smaller decreases. Lead also decreased in all treatments, with the most pronounced reductions observed in the SFD+NPK and LFD+NPK treatments.

Chromium changes were small across treatments, with slight increases observed in the SFD and LCM treatments and small decreases in the remaining treatments.

Cadmium showed minimal variation among treatments, with very small decreases observed across all treatments and no statistically meaningful differences.

Overall, changes in heavy metal concentrations were small in magnitude and generally fell within a narrow range across treatments.

Table 18. Mass balance of nutrients and heavy metals based on fertilizer inputs and crop removal for 2018-2019

	C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Fertilizer input	0.00	9.90	57.59	46.94	34.44	31.47	8.61
Plant uptake	46.54	58.33	55.78	75.68	72.12	69.28	94.70
Mg balance kg ha^{-1}	-46.54^{bc}	-48.43^{bc}	1.80^{a}	-28.75^{ab}	-37.69^{ab}	-37.81^{ab}	-86.09^{c}
Fertilizer input	0.00	51.86	299.89	87.50	68.01	59.12	17.00
Plant uptake	56.52	80.09	70.72	68.09	73.48	72.00	85.94
Ca balance kg ha^{-1}	-56.52^{e}	-28.23^{d}	229.18^{a}	19.40^{b}	-5.47^{c}	-12.88^{cd}	-68.93^{e}

	C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Fertilizer input	0.00	0.00	1.59	0.97	1.18	0.36	0.30
Plant uptake	1.53	1.50	1.44	1.46	1.27	1.47	1.54
Fe balance mg kg^{-1}	-1.53^{c}	-1.50^{c}	0.15^{a}	-0.49^{b}	-0.09^{ab}	-1.12^{c}	-1.24^{c}
Fertilizer input	0.00	0.00	0.32	0.39	0.23	0.10	0.06
Plant uptake	0.24	0.30	0.26	0.28	0.28	0.31	0.31
Mn balance mg kg^{-1}	-0.24^{cd}	-0.30^{d}	0.06^{a}	0.11^{a}	-0.05^{b}	-0.22^{c}	-0.25^{cd}
Fertilizer input	0.00	0.00	0.29	0.15	0.16	0.04	0.04
Plant uptake	0.17	0.18	0.19	0.19	0.17	0.17	0.18
Zn balance mg kg^{-1}	-0.17^{e}	-0.18^{e}	0.10^{a}	-0.04^{c}	-0.01^{b}	-0.14^{d}	-0.14^{d}
Fertilizer input	0.000	0.000	0.207	0.115	0.118	0.029	0.030
Plant uptake	0.015	0.030	0.022	0.022	0.026	0.030	0.029
Cu balance mg kg^{-1}	-0.015^{d}	-0.030^{e}	0.185^{a}	0.093^{b}	0.092^{b}	0.002^{c}	0.001^{c}
Fertilizer input	0.00	0.00	8.51	9.32	5.84	2.33	1.46
Plant uptake	13.35	27.58	23.73	24.41	25.48	22.79	36.63
Ni balance mg kg^{-1}	-13.35^{a}	-27.58^{c}	-15.22^{ab}	-15.09^{ab}	-19.65^{b}	-20.46^{b}	-35.17^{d}
Fertilizer input	0.00	0.00	0.69	0.58	0.55	0.14	0.14
Plant uptake	18.63	24.44	25.56	26.42	40.55	51.40	45.23
Pb balance mg kg^{-1}	-18.63^{a}	-24.44^{a}	-24.88^{a}	-25.84^{a}	-40.00^{b}	-51.26^{b}	-45.09^{b}
Fertilizer input	0.000	0.000	0.096	0.098	0.038	0.025	0.009
Plant uptake	0.040	0.048	0.039	0.032	0.036	0.037	0.042
Cr balance mg kg^{-1}	-0.040^{ef}	-0.048^{f}	0.057^{b}	0.067^{a}	0.001^{c}	-0.013^{a}	-0.033^{e}
Fertilizer input	0.00000	0.00000	0.00037	0.00017	0.00015	0.00004	0.00004
Plant uptake	0.005	0.006	0.006	0.005	0.005	0.006	0.008
Cd balance mg kg^{-1}	-0.005^{a}	-0.006^{a}	-0.005^{a}	-0.005^{a}	-0.005^{a}	-0.006^{a}	-0.008^{a}

Note. Different letters indicate significant differences between treatments ($p < 0.05$); non-letters indicate no significant difference. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.3.7. Chemical properties of maize grains

Table 18 shows the chemical properties of maize grain (protein, zein and starch content) in different fertilizer treatments. The statistically highest protein content was found in the NPK+CAN and SFD+NPK treatments and the lowest concentration in the C treatment. In addition, the statistically highest zein content was found in NPK+CAN, LCM and SFD+NPK. The starch content was also statistically highest in the NPK+CAN, LCM, LFD and LFD+NPK treatments.

Table 19. Chemical properties of maize grain

Treatment	Protein %	Zein g kg^{-1} DM	Starch g kg^{-1} DM
C	6,8^{b}	28,8^{b}	718,4^{c}
NPK+CAN	8,3^{a}	48,2^{a}	732,1^{abc}
LCM	7,3^{b}	44,7^{a}	732,8^{abc}
SFD	7,1^{b}	40,1^{ab}	721,4^{bc}
LFD	7,3^{b}	38,6^{ab}	746,8^{a}
SFD+NPK	7,6^{ab}	42,4^{a}	725,5^{bc}
LFD+NPK	7,4^{b}	41,1^{ab}	736,0^{ab}

Note. Letters (a, b and c) indicate significant differences between groups for 2018 ($p<0.003$) and for 2019 ($p<0.79$). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

The maize grain yield was positively correlated with the zein concentration (67.9 %) in all treatments. Table 5 shows the correlation for each treatment separately. Additionally, it shows which of the treatments had the strongest correlation between maize grain yield and zein. The MF+LFD (99.6 %), MF (90.7 %) and LFD (88.0%) treatments showed the strongest and highest positive correlation between maize grain yield and zein, while the lowest correlation was found for C treatment (2.9 %).

Table 20. Correlation of maize grain yield and zein between treatments

Treatment	C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Grain yield and zein correlation %	2.9	90.7	42.8	34.6	88.0	32.8	99.6

Note. Letters (a, b and c) indicate significant differences between groups for 2018 (p<0.003) and for 2019 (p<0.79). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

As shown in Figure 24 nitrogen content was strongly and positively (<.0001) correlated with the concentration of proteins (99.93 %) and zeins (73.91 %) in the grains in all treatments, while starch gave negative correlation with N content.

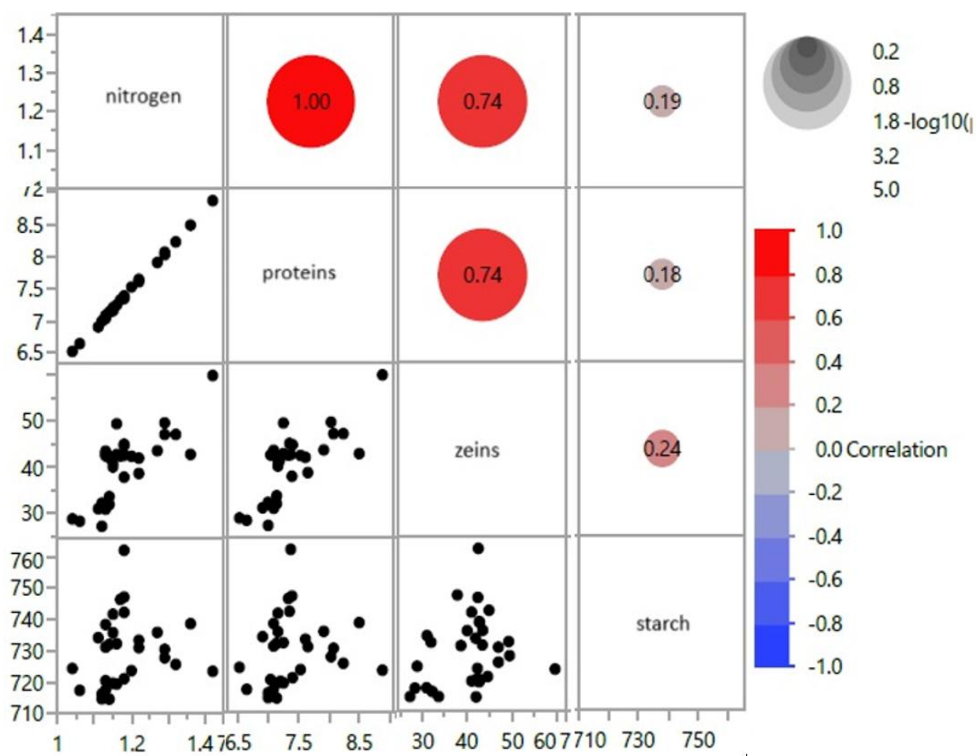

Figure 24. Correlation between N, proteins, zeins and starch as scatterplot matrix

4.4. LABORATORY EXPERIMENT

The results showed a statistically significant interaction between time and fertilizer treatment for the NH_4^+−N, NO_3^-−N and Nmin content and N release (p<.0001).

On average, statistically highest NH_4^+–N content was observed on day 0, while the lowest content was observed on day 80. Regarding the treatments, NPK+CAN had statistically highest average NH_4^+–N content, followed by LFD+NPK<SFD+NPK<LFD<LCM<C<SFD.

As for the NO_3^-–N content, the highest average value was measured on day 120 and the lowest on day 0, as expected. As for the treatments, the highest average NO_3^-–N content was observed in the NPK+CAN treatment, followed by LFD+NPK. The lowest content was observed in the control treatment (C), where no fertilization was applied (NPK+CAN<LFD+NPK<SFD+NPK<LFD<LCM<SFD<C).

On average, the total mineral N content was statistically highest on day 120 and lowest on day 0. No significant differences were found on days 40, 60 and 80. Once again, the highest average Nmin content was found in the NPK+CAN treatment, followed by the LFD+NPK<SFD+NPK<LFD<LCM<SFD<C treatment.

On average, there was no statistically significant difference in N release between day 60 and 120, while the lowest value was observed on day 0. Regarding the treatments, no significant difference was found between the control (C) and the SFD treatment. The highest N release was again observed in the NPK+CAN treatment, followed by LFD+NPK<SFD+NPK<LFD<LCM<SFD<C treatment.

4.4.1. N dynamics in the soil

At the begining of the experiment, all treatments provided significant amounts of N to the soil in the form of NH_4^+−N (Figure 24). NPK+CAN, LFD and LCM tratments gave highest amount of NH_4^+−N at the begining of incubation compared to other ones. Up to day 20, there was a slightly build up of NH_4^+−N in the C treatment (unfertilized soil) and on SFD treatment compared to other treatment where NH_4^+−N dropped and keep droping up today 100. Again, up to day 120, all treatments slightly increased (Appendix, Table 1 and 2).

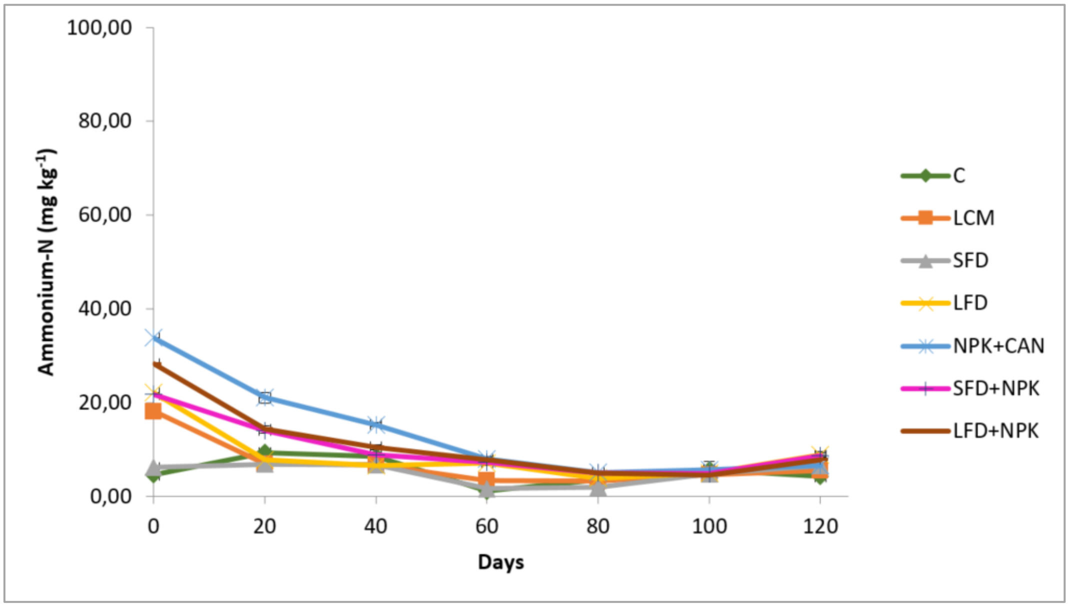

Figure 25. Ammonia content derived from different fertilizer treatments during incubation period of 120 days

Note. Error bars indicate standard deviations, where absent, (n=3). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

The NO_3^-−N content increased during the incubation experiment through whole 120 days in all treatments. NPK+CAN, LFD+NPK and SFD+NPK gave highest increase in NO_3^-−N content up to day 120 (Figure 25) (Appendix, Table 1 and 2).

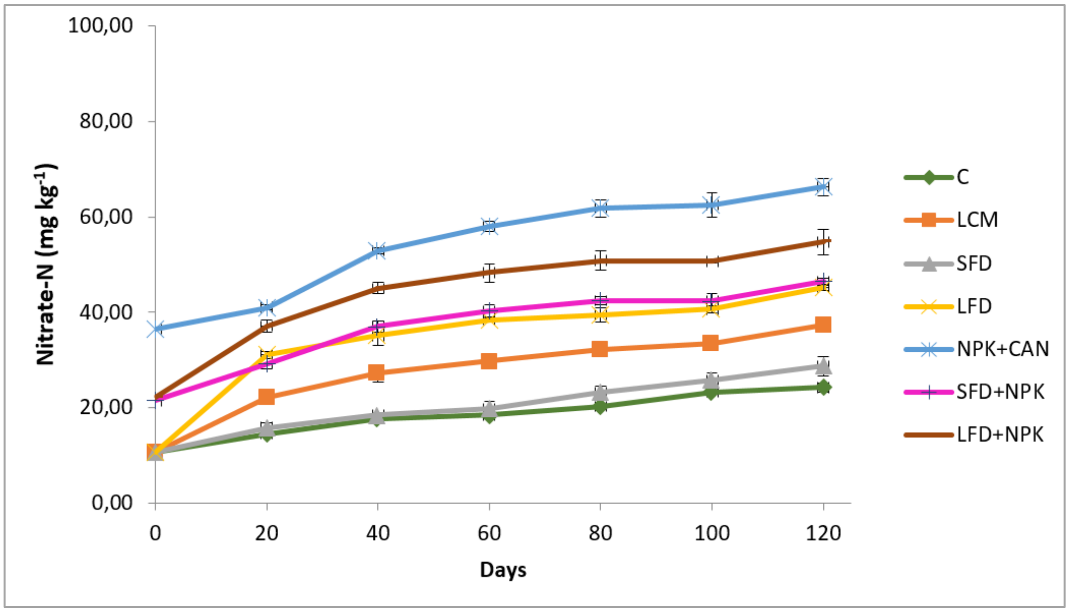

Figure 26. Nitrate content derived from different fertilizer treatments during incubation period of 120 days

Note. Error bars indicate standard deviations, where absent, (n=3). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

The trend of NO_3^-−N growth was similar for all treatments to that of total mineral N (Figure 26), showing that any NH_4^+−N was quickly nitrified to NO_3^-−N (De Neve et al., 2004; Sigurnjak et al., 2017) (Appendix, Table 1 and 2).

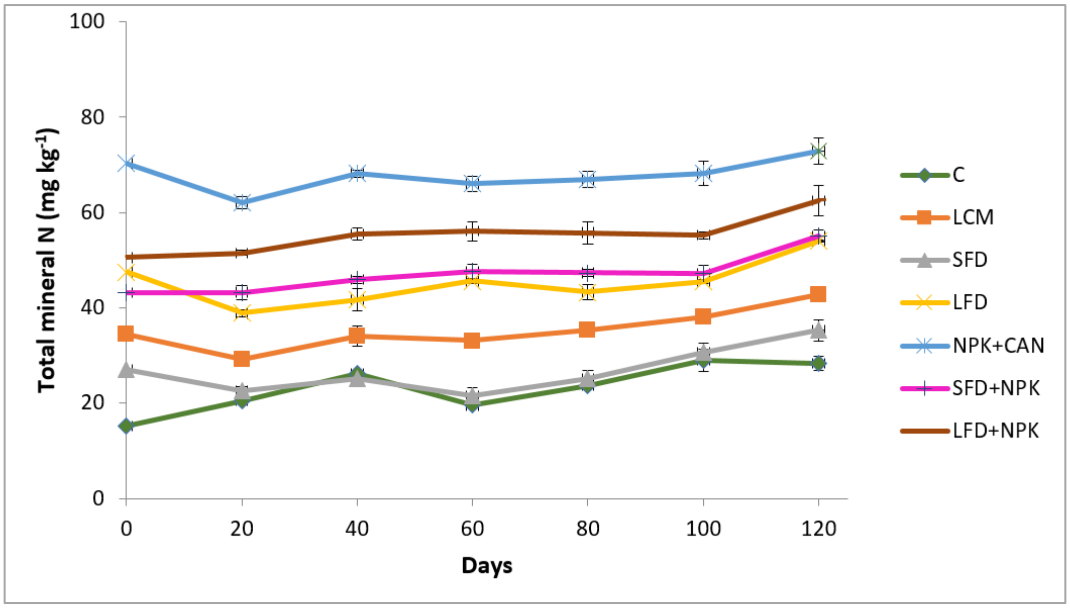

Figure 27. Evolution of mineral N (mg kg^{-1}) in the non treated and treated soil with different fertilization treatments (n=3) during an incubation experiment of 120 day in total

Note. Error bars indicate standard deviations, where absent, (n=3). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

4.4.2. N release and N mineralization

Figure 27 shows the N release ($N_{rel,net}$; %) from the tested fertilizers in a 120-day incubation experiment. In the initial phase of the experiment, the treatments using LFD and NPK+LFD showed an increase in $N_{rel,net}$ along with the C treatment observed on day 20. The remaining treatments, NPK+CAN, LCM, SFD and SFD+NPK, showed a reduction in $N_{rel,net}$ on day 20. The immobilization of N in the treatments where a reduction occurred was probably due to the start of the incubation experiment and the soil treatment (drying and wetting) (De Neve et al., 2003). Over time, from day 20 to day 60, $N_{rel,net}$ increased in all treatments up to this point. The treatments NPK+CAN (90±3) and LFD+NPK (71±4) produced the highest $N_{rel,net}$ on day 60. After day 60 and up to day 100, all treatments except SFD and C had a decrease in $N_{rel,net}$. After day 100, $N_{rel,net}$ began to increase again until day 120, with the highest Nrel,net on day 120 for the NPK+CAN treatment (87±5), followed by LFD+NPK (66±6). Comparing all treatments with NPK+CAN, a reference treatment with 100 % release from the beginning, the treatment LFD+NPK was 20 %, SFD+NPK 36 % and LFD 37 % lower than NPK+CAN. The lowest $N_{rel,net}$ in the entire incubation experiment was observed in the LCM, SFD and SFD+NPK treatments and in treatment C, in which no N was applied. On day 120, the $N_{rel,net}$ was 27±1 for LCM, 13±4 for SFD, 51±1 for SFD+NPK and 2.2±0 or the C treatment.

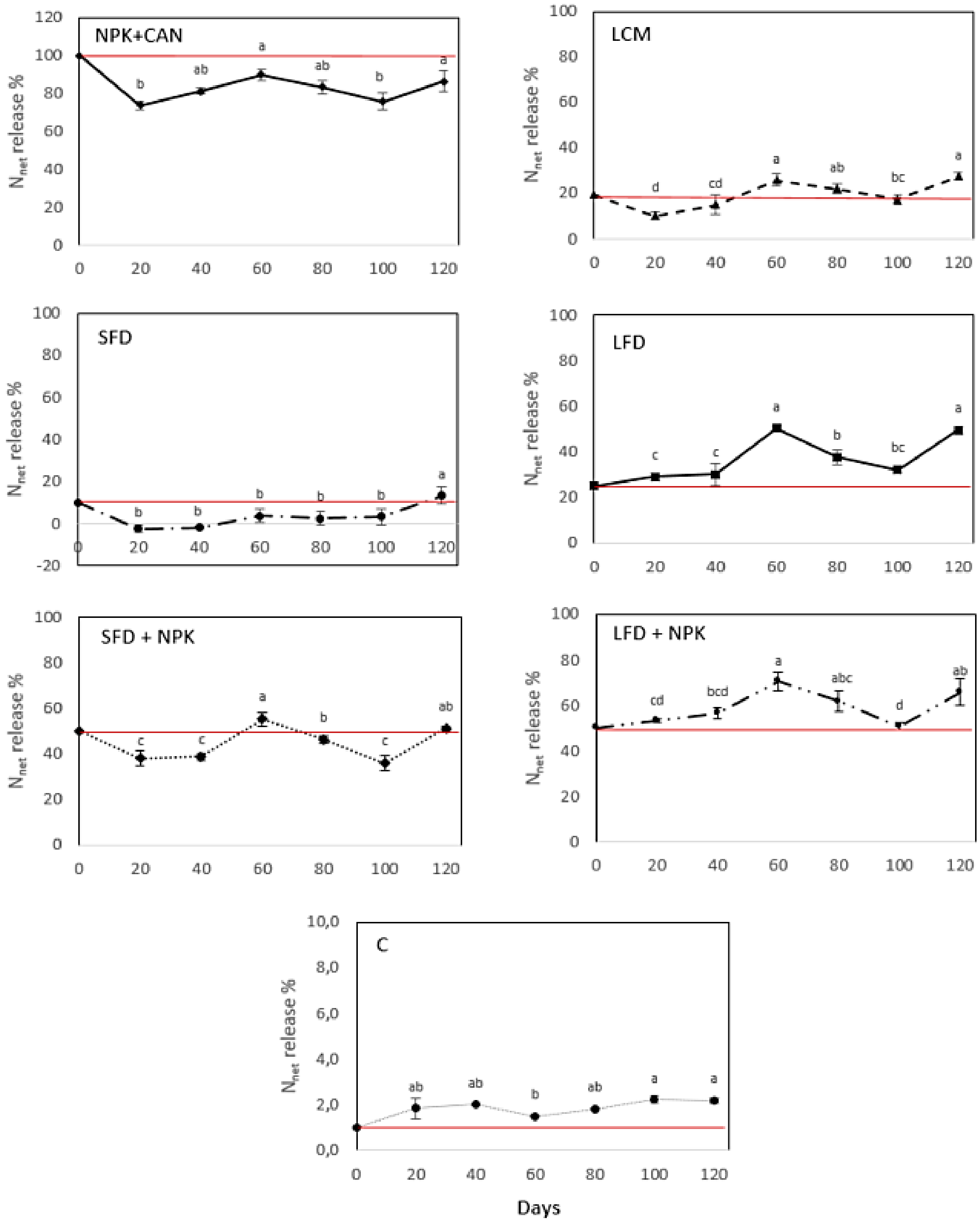

Figure 28. N release ($N_{rel,net}$; %) relative to the N input of added fertilizers in 120-day incubation experiment with the unfertilized treatment

Note. Value plotted at t=0 indicates the percentage of mineral N in applied material and is presented with straight line throughout 120 days of incubation time. Values observed above the line indicate net N mineralization, while values below the line indicate net N immobilization. Error bars indicate standard deviations (n=3). 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

The $N_{min,net}$ (expressed as % of organic N applied) from amended treatments on day 120 was 0%, -6%, 9% 10%, 13%, 16% and 33% for NPK+CAN, SFD+NPK, LFD+NPK, LCM, SFD, SFD+LFD, LFD, respectively (graph not shown). Obviously, the amount of applied organic N differed greatly between tested materials and was mostly low, which lead to large variabilities in the estimation of $N_{min,net}$ data.

4.5. STATISTICAL COMPARISON BETWEEN LABORATORY AND FIELD DATA

To evaluate N transformations under controlled and natural conditions, Pearson correlations and simple linear regressions were performed on data from both laboratory and field experiments.

4.5.1. Laboratory correlations (0−30 cm)

In the laboratory experiment, Total N showed a significant positive correlation with NH_4^+ (r=0.660, p=0.001). Correlations between Total N and NO_3^- (r=0.152, p=0.510) and between NH_4^+ and NO_3^- (r=0.247, p=0.280) were not significant.

4.5.2. Field correlations (0−30 cm)

In the field experiment at 0−30 cm, NH_4^+ and NO_3^- were strongly correlated (r=0.917, $p < 0.001$). Total N correlated significantly with NO_3^- (r=0.575, p=0.006), while the correlation with NH_4^+ was moderate and not significant (r=0.419, p=0.059).

4.5.3. Laboratory–field cross-correlations (0−30 cm)

Correlations between laboratory and field datasets at 0–30 cm were weak and not statistically significant, including Total N in the laboratory−Total N in the field (r=0.380, p=0.090), NH_4^+ in the laboratory− NH_4^+ in the field (r=−0.267, p=0.242), and NO_3^- in the laboratory− NO_3^- in the field (r=0.089, p=0.701).

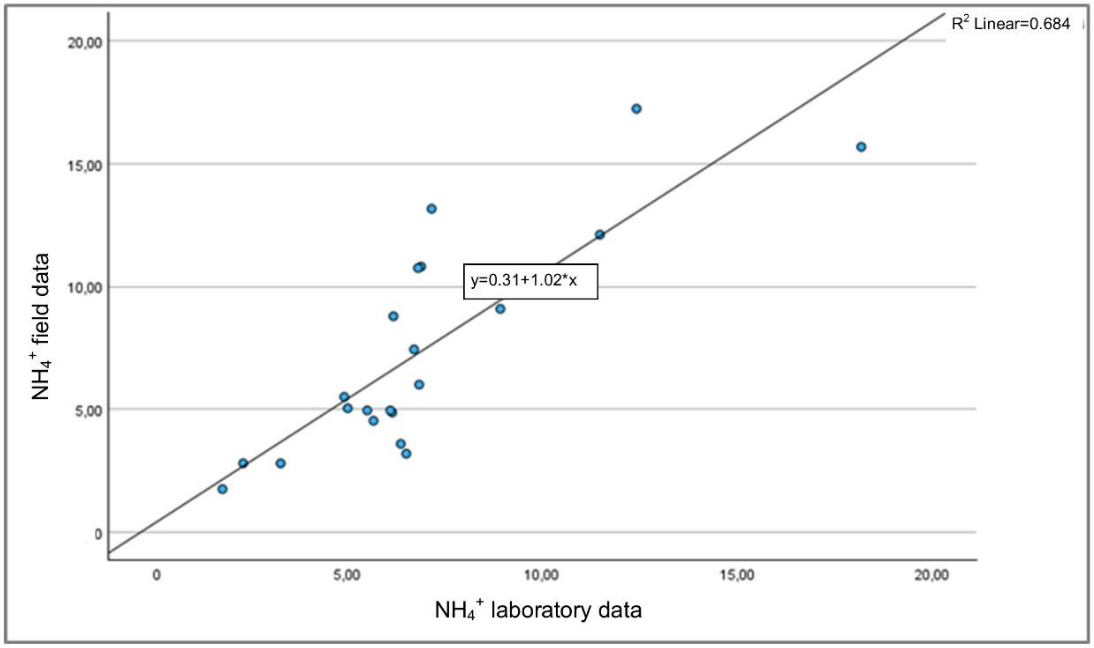

Figure 29. Correlation of NH_4^+ concentrations between field and laboratory data (0−30 cm)

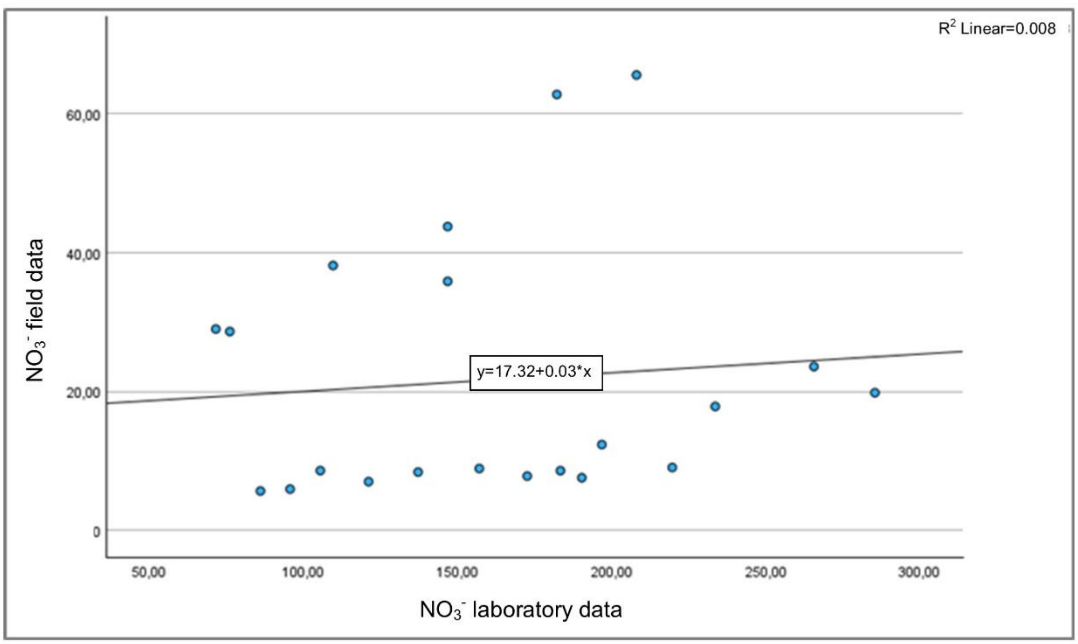

Figure 30. Correlation of NO_3^- concentrations between field and laboratory data (0−30 cm)

4.5.4. Field correlations at greater depth (0−90 cm) compared with laboratory (0−30 cm)

When field depth was extended to 0−90 cm, NO_3^- showed a strong correlation with laboratory NO_3^- ($r=0.739$, $p<0.001$).

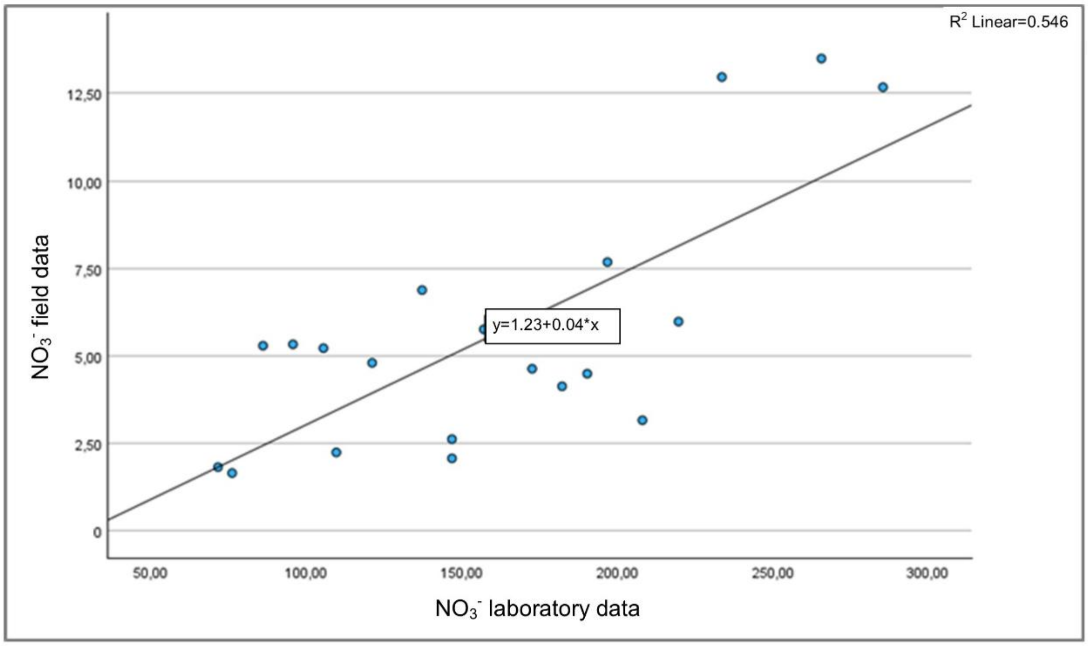

Figure 31. Field correlations of NO_3^- at greater depth (0−90 cm) compared with laboratory (0−30 cm)

4.5.5. Regression analysis: temperature and N forms

Simple linear regression analyses were conducted to assess the effect of soil temperature on mineral N forms in the field experiment. Temperature did not significantly predict NH_4^+ concentrations ($R=0.309$, $R^2=0.096$, $F_{1,19}=2.008$, $p=0.173$). The regression coefficient was negative ($B=-0.473$), indicating a weak decreasing trend of NH_4^+ with increasing temperature, with temperature explaining 9.6% of the observed variability. Similarly, temperature showed no significant relationship with NO_3^- concentrations ($R=0.107$, $R^2=0.011$, $F_{1,19}=0.219$, $p=0.645$). The regression coefficient for NO_3^- was also negative ($B = -0.677$), and temperature accounted for only 1.1% of the variation in nitrate concentration.

Table 21. Summary of significant correlations and regression analyses for soil N forms

Variable Relationship	r / R^2	Significance	Interpretation
Total N laboratory − NH_4 laboratory	0.660	<0.001 **	Strong positive (mineralization)
NH_4 field − NO_3 field	0.917	<0.001 **	Very strong positive (nitrification)
NO_3 laboratory − NO_3 field (0−90 cm)	0.739	<0.001 **	Strong link (nitrate leaching)
Temperature → NH_4 field	R^2=0.096	p = 0.173	Weak, not significant
Temperature → NO_3 field	R^2=0.011	p = 0.645	Negligible effect

4.5.6. Relationship between laboratory incubation–derived N availability and field N response across fertilization treatments

Linear regression analysis (Figure 32) revealed a strong and statistically significant relationship ($p < 0.001$) between N_{min} measured in laboratory incubation (0–30 cm) and N availability observed under field conditions, with incubation-derived values explaining 92% of the variability among treatments ($R^2 = 0.92$). The fitted model ($y = 0.546x + 124.46$) indicates that field N availability increased proportionally with N_{min} released during incubation. However, the slope less than one shows that each unit increase measured in the incubation corresponded to a smaller increase under field conditions. Along with the relatively high intercept, this suggests that laboratory incubation effectively captures relative differences among fertilization treatments but underestimates absolute N availability due to additional field-level N sources not represented in the incubation, particularly ongoing mineralization and N from soil layers below 30 cm (30-60 and 60-90 cm). This pattern was consistent across treatments, as laboratory and field measurements showed a similar ranking of N availability.

NPK+CAN exhibited nearly identical values in both approaches, reflecting its rapid and predictable N supply, whereas organic amendments showed larger variations. Higher field values for LCM suggest continued mineralization and/or downward redistribution of Nmin, while SFD displayed low N availability in the incubation, likely due to its low initial NH4+-N/Ntotal ratio and limited short-term mineralization. Combined organic and mineral treatments (SFD+NPK and LFD+NPK) reduced these discrepancies, indicating a more synchronized N supply under field conditions.

Table 22. Comparison of nitrogen availability estimated from laboratory incubation and measured under field conditions across fertilization treatments (0–30 cm)

kg ha^{-1}							
Treatments	C	NPK+CAN	LCM	SFD	LFD	SFD+NPK	LFD+NPK
Laboratory experiment	119	306	179	148	227	231	263
Field experiment	185	291	223	216	230	248	282

Note. 1-unfertilized control (C); 2- mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

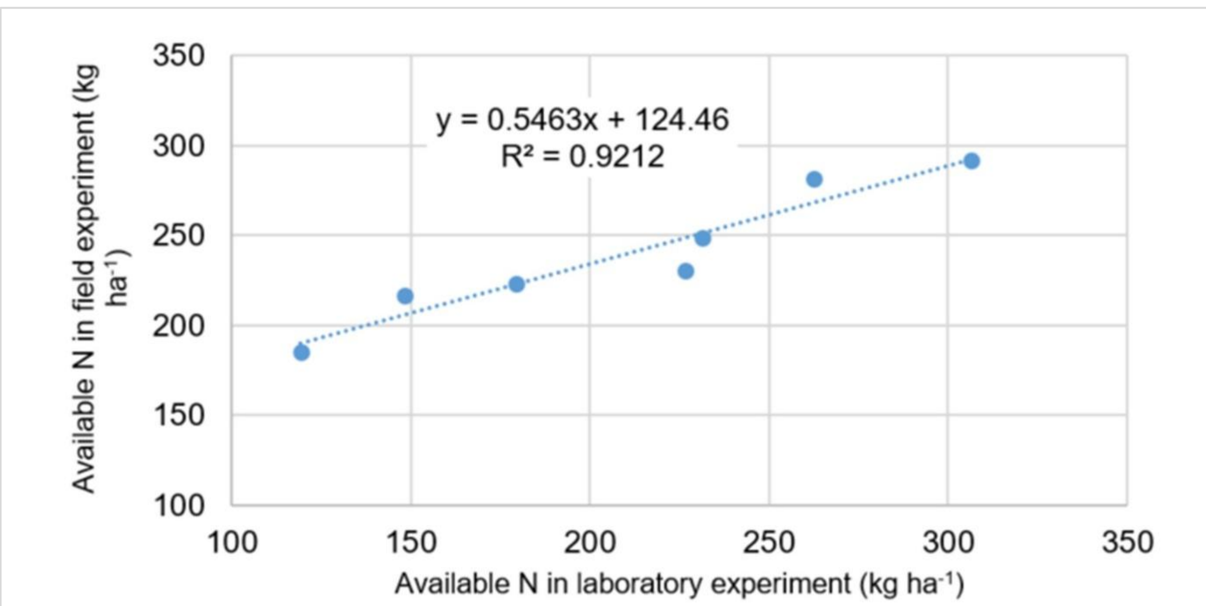

__Figure 32.__ Linear relationship between incubation-derived mineral nitrogen and field nitrogen availability across fertilization treatments

5. DISCUSSION

5.1. Field experiment

The main goal of this experiment was to determine the effects of digestate fertilization as a partial or complete replacement of mineral fertilizers on the yield and quality of maize and to determine the degree of contamination of the soil-plant system with harmful inorganic pollutants (heavy metals and nitrates). For this purpose, the data collected in two consecutive vegetation years were processed using analysis of variance (ANOVA). The influence of the fertilizer treatments on the yield and quality of the maize grain is analysed using ANOVA, with the fertilizer treatments treated as independent variables and the replicates as random variables. The effects of fertilizer treatments on nitrogen (N) mineralisation in the laboratory experiment and on N uptake, NFRV, heavy metals, nitrates and ammonia dynamics in the field experiment are analysed using ANOVA, with fertilizer treatments and sampling time treated as independent variables and replicates as random variables.

In addition to agronomic and environmental performance, the results are interpreted within the context of relevant legislative frameworks regulating nutrient management and soil protection. The findings are also discussed with reference to the EU Nitrates Directive (91/676/EEC), the RENURE criteria for the use of recovered N from manure and digestate, and the Croatian Ordinance on the Protection of Agricultural Land from Contamination by Pollutants (NN 71/2019). These legislative frameworks establish threshold values and management principles for the application of N, nitrate losses, and the accumulation of heavy metals in agricultural soils and crops. Therefore, they serve as important guidelines for evaluating the suitability and environmental safety of digestate-based fertilization strategies.

5.1.1. Soil and plant characteristics

Soil pH is a key factor when it comes to how easily plants can absorb nutrients, so even small changes can have a big impact on soil fertility. At the Maksimir experimental site, the initial soil pH measured in water (pH_{H_2O}) was moderately acidic -

5.47 in 2018 and 5.26 in 2019. When measured in potassium chloride (pH_{KCl}), which gives a better sense of the active acidity of the soil, the values were even lower: 4.21 and 3.93 respectively. According to Thun's classification, this puts the soil in the strongly acidic category. As the experiment progressed, the pH values continued to decrease in all treatments, indicating that the soil became increasingly acidic over time (see Table 1). Glowacka et al. (2020) had a similar experiment on soil where pH_{KCl} was 4.40. In her study, pH was increased from very acidic to acidic after digestate use. This was not the case in our study in 2018, as the pH value decreased in all treatments. The smallest decrease was recorded in the LFD and LFD+NPK treatments. In the second year, a different scenario occurred, where pH increased in all treatments, most significantly and highest in the LFD and LFD+NPK treatments (increase of 0.32 and 0.36 of pH, respectively) which allows us to confirm that digestate, especially the LFD, has a positive effect on increasing the pH of the soil. Even if it is a small decrease in the soil, it is still important as it favors plant better development. This was also the case with Koszel and Lorencowicz (2015), where the pH value of the soil increased slightly from 7.56 to 7.63 after the use of digestate. Many studies have shown that the optimal pH value for normal growth of maize ranges from 5.51 to 7.20, depending on the variety of maize (Walter et al., 2000; Happy Msawika, 2016; Zhang et al. 2022; Mandić et al., 2023).

Similar patterns have been observed in other studies, Ren et al. (2020), for example, found that applying higher levels of digestate led to a slight but significant decrease in soil pH. This is because digestate contains a lot of ammonium N, which can temporarily raise the pH, but eventually lowers it during the nitrification process what was the case in this study in all treatments. In addition, when plants take up ammonium, they release hydrogen ions into the surrounding soil, which further increases acidification (Smith & Read, 2008). This shift in pH can also affect soil microbes (Pan et al., 2020). The application of digestate during the study had a different effect on the soil pH, as has also been demonstrated in other studies (Przygocka-Cyna & Grzebisk, 2020). It is assumed that the changes in soil pH depend not only on the application of digestate in this case, but also on other environmental factors.

The N content in the soil measured in two consecutive years shows that the soil is well supplied with total N according to Woltmann (Škorić, 1965). The values measured at the Maksimir site for the initial state of the soil were 0.14 % N in 2018 and 0.13 % N in 2019. The N content fluctuated between 0.12 and 0.14 % during the experiment within three soil samplling moments and different fetilization treatments. Čoga et al. (2006) state that such a soil is classified as a well supplied soil with total N. Šimon et al. (2015) in his experiment contained total N in range 0.15-0.16 % (application of digestate, digestate+straw, NPK, cattle slurry and no fertilization), while Sigurnjak et al. (2017) depending on treatments, determined total N in the soli in range from 0.10-0.11% (animal manure AM+CAN, AM+mineral concentrate MC, CAN, MC, liquid fraction of digestate and unfertilized control). It is worth to remind that N occurs in the soil in organic and mineral form. Organic N is not directly accessible to plants, but only after complex N compounds have been broken down to ammonia through mineralization processes (Herak Ćustić et al., 2025).

Regarding total N in the maize, Bergmann (1992) states that the optimum N concentration for maize is between 3.5 and 5.0 % at fully developed leaves (40-60 cm plant height). In our study, this was the V4 stage of maize. In this stage, the total N content ranged between 3.44 and 4.14 % in 2018 and between 3.36 and 3.78 % in 2019, which was consistent with the values reported by Bergmann (1992) for the total N content in maize. In the later stages, R5 and R6, the N content decreased three times in both years. Comparing the data with the N content in the plant during the vegetation period, in both years the highest N content in the plant was found in the vegetative phase V4, after which the dynamics of the N content decreases in the reproductive phase R5 (next sampling) and in the reproductive phase R6. This pattern also aligns with findings by Hammad et al. (2017), who reported that N concentration in maize roots is typically higher during early growth and declines toward maturity as N uptake and assimilation increase. Accordingly, the significant decrease in nitrate levels later in the season in our study reflects active N absorption by the plants, supporting their biomass and grain development. Although the highest N content was observed in the treatment with mineral fertilization only (NPK+CAN), comparable results were also obtained in treatments combining digestate fractions with mineral fertilizers (SFD+NPK and LFD+NPK). These results indicate that similar nutrient availability can be achieved by partially replacing mineral fertilizers with digestate fractions (SFD or LFD). Such an approach offers a double benefit: most probably it can reduce dependence on mineral fertilizers and thus lower fertilizer costs, while contributing to the reduction of greenhouse gas emissions and promoting more sustainable agricultural practices.

Reuland et al. (2021), based on a large European dataset, showed that only a limited proportion of digestates can be classified as mineral fertilizer-like under the RENURE framework. On average, digestates had Nmin/TN ratios of about 58% and TOC/TN values near 3.9, indicating that most materials still function primarily as organic N sources rather than true mineral fertilizers. Our results from Croatian digestates are broadly consistent with these findings but show a clearer differentiation between individual fractions. LCM and the SFD were consistently dominated by organic N, with NH_4^+–N accounting for only 5–33% of total N and relatively high and variable C/N ratios, indicating slower N mineralization and limited fertilizer-like performance. In contrast, the LFD, especially in 2019, contained a much higher proportion of mineral N (NH_4^+–N/total N=0.43). Although this value remained below the RENURE threshold, it approached the upper range reported by Reuland et al. (2021), who found that only about 5% of digestate samples reached Nmin/TN ≥ 90%. This intermediate N composition aligns with field-scale observations in the literature, where digestates containing both organic and mineral N typically show slower nitrification, lower short-term nitrate availability, and in some cases reduced nitrate leaching compared with mineral fertilizers. Importantly, despite variability in N form and soil N dynamics, contaminant concentrations were not a limiting factor in either study. Similar to the European dataset, in which about 95% of samples met copper (Cu) and zinc (Zn) thresholds, all Croatian digestate fractions remained well below national regulatory limits (as explained further in the discussion). Overall, these results reinforce the view that RENURE suitability is determined mainly by N form, C/N (or TOC/TN) ratio, and the predictability of nitrate behavior at the field scale, rather than by heavy metal concentrations. In this sense, RENURE offers an opportunity to enhance circular nutrient management, but it also requires careful implementation to ensure compliance with the water protection goals of the Nitrates Directive.

According to Bergmann (1992), nitrate levels in plants can vary considerably without causing visible signs of plant damage, depending on factors such as the species and organ affected, the availability of N and nitrate and environmental conditions such as precipitation, temperature and especially light intensity. In our study, nitrate concentrations were higher in the early phase of sampling (V4) and decreased in the later phase (R5), following a similar pattern to that described by Maynard et al. (1976), who found that nitrate concentrations vary between plant parts and change as the plant matures. In addition, nitrate accumulation is influenced by genetic characteristics, the nitrate supply capacity of the soil and environmental conditions during the growing season, such as the drought recorded in August in our study. Although the highest nitrate concentrations in both years were observed in the NPK+CAN, SFD+NPK and LFD+NPK treatments, no negative effects on plant health were observed. The treatments with digestate fractions had no negative effects on the plants, neither in terms of total yield nor nitrate accumulation.

During a a season with favorable agro-ecological conditions, nitrate taken up by plants is rapidly reduced to amino acids and subsequently incorporated into proteins, a process that occurs mainly in green leaf tissue. This conversion is generally efficient enough to prevent toxic nitrate accumulation. However, under conditions such as prolonged drought, high temperatures and low humidity, or in soils rich in nitrate or organic matter, nitrate can accumulate in plant tissue in higer amounts (Steinke, 2012). In our study, it is assumed that despite some periods of environmental stress (drought), the plants effectively bild in nitrates into amino acids and proteins, preventing toxic accumulation and maintaining normal plant development.

All measured values of soil phosphorus (both total P and available P) were sufficient to meet the nutrient requirements necessary for the normal growth and development of maize throughout the growing season, regardless of the sampling periods. According to the classification of available P in the soil, the available P content classifies the soil as well supplied with phosphorus (Wunderer et al., 2003; Čoga et al., 2006). In 2018, in the soil was determined a slightly higher content of available P than in 2019, with an increase of 2.24 mg P_2O_5 per 100 g of soil. Eventhought there was no significant statistical differences among treatments, in both of the years LFD+NPK

(14.63 and 11.93 P_2O_5 per 100 g of soil, respectively) resulted with highest content of available P in the soil. Przygocka-Cyna and Grzebisz (2020) carried out a similar study using digestate as a treatment. In their study, they fertilized the soil with 200 kg N ha^{-1} from digestate obtained from cattle slurry and maize sillage. The available P content in the soil after the first year of application was 45.57 mg, in the second year it was 57.02 mg and in the third year 40.56 mg P_2O_5 per 100 g of soil respectively, which is three times as much as in this study. The better P availability was probably due to the higher pH in the study by Przygocka-Cyna and Grzebisk (2020) or simply due to different fertilization practices with the application of larger amounts of P in previous periods. According to Barłóg et al. (2020) and their application rate of 20 t ha^{-1} of digestate, digestate+straw, cattle slurry and NPK, during the four-year trial period, the fertilization treatments, digestate, digestate+straw, cattle slurry and NPK resulted in a slightly lower available P content (21.27; 19.85; 21.59 and 20.82 mg P_2O_5 per 100 g of soil, respectively) from our study. The P content was converted to P_2O_5 from both papers. Koszel and Lorencowicz (2015) also conducted an experiment with alfalfa in which the P_2O_5 value before the use of digestate (36 000 l ha^{-1}) was 31.40 and increased to 37.30 mg P_2O_5 per 100 g of soil after the use of digestate.

Sigurnjak et al. (2017) reported total P concentrations in soil between 0.07 % and 0.09 % in all treatments, with no statistically significant differences observed. Similarly, in the present study, soil total phosphorus concentrations ranged between 0.08 % and 0.10 % in both experimental years and remained relatively stable throughout the growing season, with no significant differences observed between treatments.

Total P concentrations in maize plants in 2018 (0.26–0.30 %) and 2019 (0.25–0.35 %) were below the lower threshold of the optimal range recommended by Bergmann (1992) (0.35-0.60%) for growth stage V4, which corresponds to the sampling period of this study. As the growing season progresses, the total P content in the plant generally decreases in R5 and R6 stage. In addition, the acidic soil reaction probably contributed to the lower phosphorus availability for the plants.

Lower pH values in the soil increase the strength of the bond between phosphate ions and the adsorption complex, which reduces the availability of P to plants. In addition, in highly acidic soils such as those in this study, P can form insoluble compounds with iron (Fe), manganese (Mn) and aluminum (Al), further limiting its availability. Another important factor influencing phosphorus availability is soil moisture, which affects both the mobility of P and the rate of replenishment of phosphoric acid in the soil solution (Figure 33).

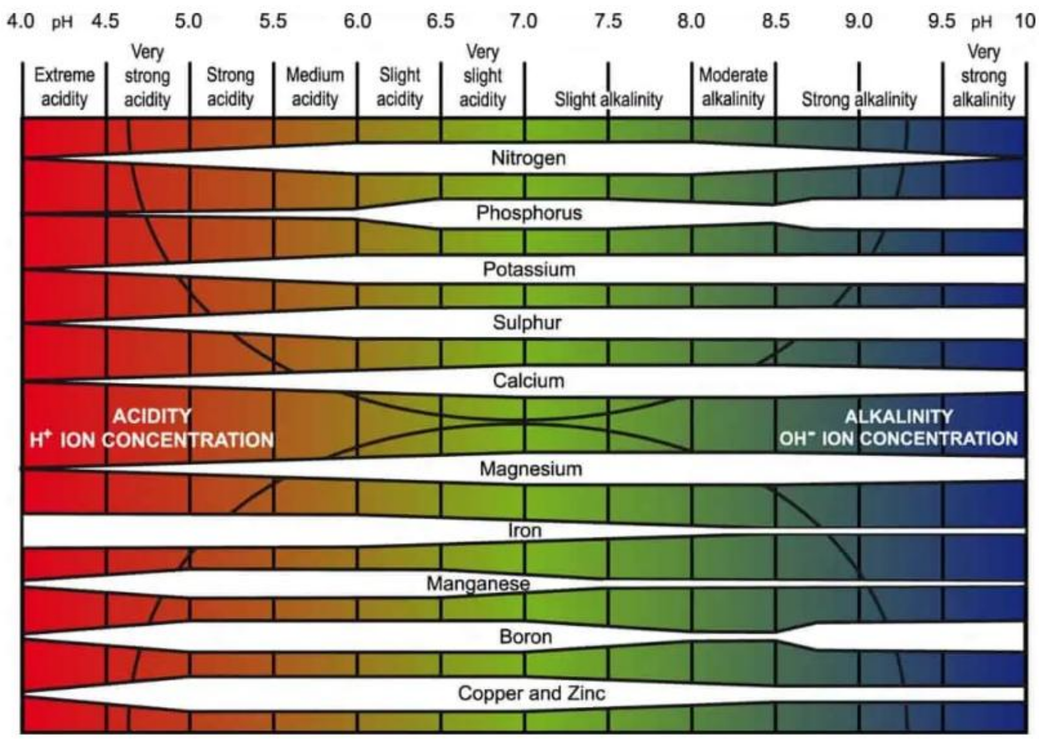

Figure 33. The availability of different nutrients at the different pH bands is indicated by the width of the white bar: the wider the bar, the more available is the nutrient (source: redrawn for PDA from Truog, 1946)

The uptake of P by plants is an active process that is particularly crucial in the early stages of development due to the high demand for this nutrient (Čoga et al., 2006; Herak Ćustić et al., 2025). Although the soil exhibited an acid reaction that limited the complete decomposition and availability of total P and potassium (K) (Čoga et al., 2006), it is assumed that this limitation did not significantly affect the final maize yield.

Moreover, the positive effect of digestate fertilization on a slight increase in the concentration of plant-available P and K compared to mineral fertilization (NPK+CAN) indicates that fertilization in combination with digestate (SFD+NPK adn LFD+NPK) can be a high-quality substitute for partial fertilization with mineral fertilizers, which can significantly reduce fertilization costs and greenhouse gas emissions.

As for the K in the soil (both total K and available K), concentrations in both years were sufficient to meet the nutrient requirements necessary for the normal growth and development of maize throughout the growing season. According to the classification of available soil K, the soil in this study is well supplied with K (Wunderer et al., 2003, Čoga et al., 2006). In 2018, the soil had a higher content of available K, 3.13 mg K per 100 g of soil more than in 2019. Additionally, LFD+NPK treatment resulted in the highest available K from all tratments in 2018, while SFD+NPK resulted the highest in 2019. A similar study was conducted by Przygocka-Cyna and Grzebisz (2020), who investigated the effects of digestate application on soil properties. In their experiment, the soil was fertilized with 200 kg N ha^{-1} using digestate from cattle slurry and maize silage. The available K content in the soil after the first, second and third year of application was 26.30, 25.63 and 18.14 mg K per 100 g of soil respectively — slightly higher than the values observed in our study. Barłóg et al. (2020) conducted a four-year trial in which different fertilization methods were applied at a rate of 20 t ha^{-1}, including digestate, digestate combined with straw, cattle slurry and mineral NPK fertilizers. Their results showed slightly lower available K contents (19.74 mg K_2O per 100 g of soil) because of lower digestate rate (180 l per 50 m^2 – 189 kg K ha^{-1}) compared to Przygocka-Cyna and Grzebisz (2020) with values of 28.33, 29.72, 26.82 and 27.16 mg K_2O per 100 g soil (200, 400 and 800 kg of digestate ha^{-1}) for digestate, digestate+straw, cattle slurry and NPK treatments, respectively. For reasons of consistency, the K content values reported in both studies were converted to K_2O. According to Koszel and Lorencowicz (2015) the K_2O value before the use of digestate valued 7.20 and doubled to 16.40 K_2O per 100 g of soil after the use of digestate. In their trial field, they used alfaalfa for cultivation. An increase in the macroelement content was found in the leaves of the alfalfa fertilized with digestate compared to the alfalfa fertilised with mineral fertilizers.

The total concentration of K in the soil followed a similar pattern to that of P, ranging from 0.18 to 0.25 % K in both years of the study. These values are comparable to those of Sigurnjak et al. (2017), who observed total K concentrations between 0.20 and 0.21 % K across all treatments, with no statistically significant differences.

Plant total K concentrations in maize in 2018 (3.23–4.06%) and 2019 (4.02–4.62%) were below the lower threshold of the optimal range recommended by Bergmann (1992) (4.50-5.80%) for growth stage V4, which corresponds to the sampling moment of this study. As the maize growing season progresses, the total K content in the maize generally decreases five to six times in R5 and R6 stage. Since the maize plant grows and becomes larger, the potassium is partitioned and distributed in the increasing biomass, decreasing the concentration per unit of tissue. At stages R5 and R6, much of the potassium is remobilized to support grain filling, while senescence and leaching reduce the total K content.

No statistically significant differences were found between the different treatments with regard to the total calcium (Ca) content in the soil in either year of the study. Calcium levels remained within a relatively narrow range between 0.32% and 0.42%, indicating stable conditions in the experimental plots regardless of the treatment applied. The total magnesium (Mg) content in the soil also showed a consistent statistical trend across both years and all sampling periods. This indicates that the fertilization strategies applied during the study did not significantly affect the overall availability of these two important secondary nutrients in the soil and that their levels remained within an expected and stable range throughout the experimental period. Lower concentrations of total Ca and Mg were reported in the study by Sigurnjak et al. (2017), where the values of total Ca ranged between 0.21-0.24 % and total Mg between 0.15-0.17 %.

The total Ca and Mg content of the maize corresponded to the data in the literature. Bergmann (1992) stated that the optimum Ca concentration for maize at the V4 stage is between 0.50-1.00%, while Mg is 0.20-0.30%. In our study, the range in all experimental series was 0.68-0.72% for total Ca and 0.20-0.23% for Mg. This shows that all requirements for these two elements are met.

The results shown in Table 11 and 12 indicate that the Fe content of the soil closely matches the pattern observed in the initial state in all treatments. Since the availability of Fe is influenced by the pH of the soil, more acidic soils tend to have higher concentrations of Fe ions in the soil solution. Due to its good mobility under acidic conditions, Fe can be leached from the topsoil into deeper layers, which can lead to its accumulation and the formation of impermeable or poorly permeable subsoil layers (Herak Ćustić et al., 2025). When comparing the total Fe content in the control treatment (C) with treatments containing digestate and mineral fertilizers, no significant differences were found in 2018. In 2019, however, a significant increase in Fe content was found in all treatments during the last sampling. Despite this increase, no negative effects on the soil were detected.

The amounts of total Mn in the 0-30 cm layer of soil ranged between 929-1419 mg Mn kg^{-1} in both years. In 2019, the Mn contents were slightly higher than in 2018. According to Čoga et al. (2019), the total Mn content in soils is between 200-300 mg Mn kg^{-1}, of which only 0.1 to 1.0 % Mn is available for plants. In the study by Przygocka-Cyna and Grzebisz (2018), 0.8, 1.2 and 3.2 t kg^{-1} of digestate were used. Their Mn quantities ranged from 117.1 to 127.8 mg Mn kg^{-1}, lower than our study.

According to Bergmann (1992), the optimal Mn concentration in maize during the V4 growth stage is between 40 and 100 mg Mn kg^{-1}, while Finck (1982) gives a wider range of 30 to 160 mg Mn kg^{-1}. In 2018, the Mn concentrations in the plants were above the optimum recommended by Bergmann. The highest Mn contents were measured in the treatments LFD+NPK (164.1 mg kg^{-1}), SFD+NPK (172.4 mg kg^{-1}) and NPK+CAN (160.5 mg kg^{-1}). One possible explanation for the increased Mn content in the plant tissue is the acidity of the soil. Under natural conditions, acidic soils such as those in this study tend to increase the availability and uptake of Mn by plants. However, this increase in Mn content was only observed during the V4 phase. At the end of the vegetation period, the Mn concentration in the plants had decreased to the optimal range. This was only case in 2018, while 2019 was in optimal concentrations of Mn in the plant. Therefore, despite the increased Mn concentrations in the V4 phase, there were no harmful effects on the plants in the digestate treatments.

Agricultural soils in Croatia typically contain total Zn concentrations ranging from 25 to 100 mg Zn kg^{-1}, while the proportion of plant available Zn is much lower, generally between 0 and 25 mg Zn kg^{-1} (Čoga et al., 2019). These concentrations exceed the average Zn content reported for soils in general, which ranges from 5 to 20 mg Zn kg^{-1} (Vukadinović and Vukadinović, 2011), reflecting both natural variability and the influence of agricultural practices. According to the Ordinance on the Protection of Agricultural Land from Contamination by Pollutants (NN 71/2019), agricultural soil in Croatia is considered contaminated when total Zn concentrations exceed pH dependent threshold values: 60 mg Zn kg^{-1} in air-dried soil at pH<5, 150 mg Zn kg^{-1} at pH 5–6, and 200 mg Zn kg^{-1} at pH>6 (NN 71/2019). After the application of digestate and mineral fertilizers, a decrease in the Zn content in the soil was observed in this study. It is important to emphasize that the digestate treatments had no negative impact on soil Zn accumulation through plant uptake throughout the growing season, as explained in the following paragraph. In a comparable study, Sigurnjak et al. (2017) reported lower total Zn concentrations (41.0 to 44.0 mg Zn kg^{-1}), which could be due to different soil properties, fertilization practices and application rates in their experiment. Additionally, Przygocka-Cyna and Grzebisz (2018) had similar concentrations of Zn in the soil, ranged from 44.1 to 52.6 mg Zn kg^{-1} throught three year experiment also due to different soil properties such as soil pH, organic matter content, soil texture, soil moisture and etc.

The availability of Zn in the soil depends on various conditions such as the pH value of the soil, the content of organic matter and the interaction with other minerals. In high pH (alkaline) soils, Zn often turns into insoluble compounds, making it less accessible to plants. In contrast, lower pH values (acidic soils) usually improve the availability of Zn. In addition, organic matter can contribute to Zn being in a plant-available form by forming complexes that limit its binding to soil particles (Čoga et al., 2019).

Zinc concentrations in digestate generally fall within a moderate range, with Reuland et al. (2021) reporting a mean value of 361 mg Zn kg^{-1} DW and a central value of 300 mg Zn kg^{-1} DW across a large European dataset. Elevated Zn concentrations were primarily associated with digestates derived from pig manure, reflecting the use of Zn supplements in pig feed, whereas digestates originating from cattle manure typically exhibited substantially lower levels and largely complied with regulatory thresholds as in this study. The liquid fraction (LF) of digestate showed comparable or slightly lower Zn concentrations, with median values of approximately 300–310 mg Zn kg^{-1} DW, and about 96% of LF samples complied with the RENURE limit of 800 mg Zn kg^{-1} DW. Overall, these results indicate that both whole digestate and its liquid fraction, particularly when derived from cattle manure as in this case (229 and 263 mg Zn kg^{-1} DW for LFD), pose a low risk of Zn accumulation in soils when applied according to current regulations (Reuland et al., 2021).

The Zn content in plants varies considerably depending on the species, with reported concentrations ranging from 0.6 to 83 mg Zn kg^{-1} of dry matter (Kabata-Pendias and Pendias, 2001; Vukadinović and Vukadinović, 2011). A comparison of the measured Zn concentrations in the plant tissue (Table 13 and 14) with the optimum range for maize, which according to Bergmann (1992) is 30-70 mg Zn kg^{-1}, shows that the Zn supply to the plants was within the optimum range in all treatments. This indicates that there was neither a deficiency nor an excess of Zn in the plants during the entire vegetation period. In 2018, the highest Zn concentration in the plants was observed in the NPK+CAN treatment, while in 2019 it was highest in the LFD+NPK treatment.

Copper concentrations in the soil ranged from 23.9 to 27.8 mg Cu kg^{-1} in 2018 and from 23.1 to 34.4 mg Cu kg^{-1} in 2019. According to the Ordinance on the Protection of Agricultural Land from Contamination by Pollutants (NN 71/2019), the maximum permissible concentration of Cu in soil is 60 mg Cu kg^{-1}. As the results show, this limit was not exceeded in any treatment during the entire vegetation period. A similar study was conducted by Sigurnjak et al. (2017), where different soil properties and fertilization treatments resulted in values ranging from 16.0 to 17.0 mg Cu kg^{-1}, while Przygocka-Cyna and Grzebisz (2018) reported values between 13.0 and 21.9 mg Cu kg^{-1}. These concentrations fall within the typical range of Cu content in soils, which generally varies from 5 to 50 mg Cu kg^{-1} (Čoga and Slunjski, 2018), and are well below the contamination thresholds defined by soil pH. Soils formed by the weathering of basic rocks are known to contain higher amounts of Cu (Čoga and Slunjski, 2018). Reuland et al. (2021) reported a mean Cu concentration of 102 mg Cu kg^{-1} DW and a central value of 71 mg Cu kg^{-1} DW for digestate in a large European dataset, while in our study these concentrations were higher: 139 mg Cu kg^{-1} DW in 2018 and 292 mg Cu kg^{-1} DW in 2019. Although the database was not explicitly stratified by animal species, the authors noted that elevated Cu concentrations were mainly associated with digestates derived from pig manure, while digestates from other manure types, including cattle manure, generally had lower Cu levels. Regarding the RENURE criteria of 300 mg Cu kg^{-1} DW, approximately 95% of the analyzed digestate samples met the proposed threshold (Reuland et al., 2021).

The average Cu content in plants ranges from 5 to 30 mg Cu kg^{-1} of dry matter (Kumar et al., 2021). Based on the measured values, it can be concluded that the Cu concentration in plant tissue was within the optimum range for plant growth. According to Finck (1982), the optimal Cu concentration in plants is 7–20 mg Cu kg^{-1}, while Bergmann (1992) reported an optimal range of 7–15 mg Cu kg^{-1} during the V4 growth stage. In 2018, the Cu concentrations in plant tissue were between 8.32 and 10.33 mg Cu kg^{-1} and in 2019 between 10.71 and 12.93 mg Cu kg^{-1}. Therefore, Cu concentrations in all treatments remained below the maximum permissible level and within the optimal range, indicating no harmful effects of Cu on the soil or plants.

The availability of Fe in the soil depends largely on the pH value. The more acidic the soil, the higher the concentration of Fe ions in the soil solution. In well-moistened, acidic soils, there is also a greater risk of Fe being leached into deeper layers (Čoga et al., 2019). In a study by Przygocka-Cyna and Grzebisz (2018), Fe concentrations were reported at 1021.7 mg kg^{-1} in the first year, 1000.4 mg kg^{-1} in the second, and 632.7 mg kg^{-1} in the third year of digestate application in the soil. In our experiment, the Fe concentrations remained within the optimum range for all treatments throughout the entire test period. In 2018, the last sampling showed a slightly significantly higher Fe concentration in the SFD treatment compared to the initial state of the soil. In 2019, Fe concentrations were unchanged to the end of the experiment. As the soil pH was lower in 2019 than in 2018, this probably contributed to an increased solubility of the ferrous form of iron (Fe^{2+}), which is more available to plants and their uptke but also more prone to leaching (Figure 33), while in Przygocka-Cyna and Grzebisz (2018) study pH was much higer and concentrations were lower.

According to Finck (1982), the optimal Fe concentration in maize during the V4 growth stage is between 50 and 300 mg Fe kg^{-1}. In our measurements in 2018 during the V4 stage, Fe concentrations were between 265 and 396 mg Fe kg^{-1} in all treatments. The highly acidic soil conditions likely enhanced Fe uptake, resulting in higher than optimal concentrations in several treatments, including LCM, SFD, LFD, LFD+NPK and even the control. In 2019, Fe concentrations in maize were even higher, ranging from 601 to 951 mg Fe kg^{-1}, about twice as high as in 2018. Interestingly, in both years, the control treatment had the highest Fe concentrations in plant tissue compared to the other treatments. As the growing season progressed, these values decreased significantly. Finck (1982) also states that the optimum Fe concentration in mature maize plants should be in the range of 30–50 mg Fe kg^{-1}. It is assumed that even if our study had exceeded values of Fe, often by a factor of one to two, but no harmful effects were observed, even with the increased Fe levels at the end of the experiment. However, it should be noted that persistently high concentrations of available Fe in soil, especially in acidic environments, may pose long-term ecological risks, such as altered microbial activity and nutrient imbalance, which should be monitored in future studies.

Soils typically contain between 5 and 500 mg Ni kg^{-1} according Čoga and Slunjski (2018), indicating a strong dependence of Ni content on parent material and soil properties. Nickel (Ni) is toxic to both soil and plants at concentrations above the optimum level. Čoga et al. (2019) report that the average Ni concentration in soils is around 40 mg Ni kg^{-1}, with significant variations depending on soil type. According to the Ordinance on the Protection of Agricultural Land from Contamination by Pollutants (NN 71/2019), the maximum permissible concentration (MPC) of Ni in Croatian soil is 30 mg Ni kg^{-1}. In our study, the measured concentrations of total Ni in the initial soil before the start of the trial exceeded this limit in both 2018 (32.05 mg Ni kg^{-1}) and 2019 (31.38 mg Ni kg^{-1}). During the trial in 2018, the Ni levels in the soil fluctuated slightly, but did not increase significantly and remained above the MPC (between 30.2 and 33.0

mg Ni kg^{-1}). In 2019, Ni concentrations fell below the MPA in the first and last sampling, but exceeded the original soil value in the second sampling (between 35.6 and 36.7 mg Ni kg^{-1}). As the Ni concentrations were above the MPC in both years, even before the experiment began, this indicates a geogenic and/or pedogenic origin of the Ni in the soil. Similarly, the increased total Ni content in the soil can be attributed to the proximity of the study area to a road, which is known to contribute to the accumulation of heavy metals through vehicle emissions, tire and brake wear, and road surface degradation (Wei & Yang, 2010).

The average Ni concentration in plants ranges from 1 to 10 mg Ni kg^{-1} of dry matter (Vukadinović & Vukadinović, 2011). According to Čoga et al. (2019), the harmful concentration of Ni for plants is 20 mg Ni kg^{-1}. In our study, the Ni concentrations in plant tissue did not exceed this limit in either year. In 2018, Ni levels were between 6.8 and 8.3 mg Ni kg^{-1}, while in 2019 they were about half this level at 2.2 to 4.5 mg Ni kg^{-1} in all treatments during the V4 growth stage. The highest Ni concentration was observed in the NPK+CAN treatment in 2018, while in 2019 it was recorded in the SFD treatment. In the second sampling, Ni concentrations were even lower, about half as high as the values measured during the V4 stage in 2019.

Although the Ni levels in the soil exceeded the maximum permissible concentration, the Ni content in the maize remained well below the harmful levels and did not contaminate the plant material.

The influence of digestate fertilization on the accumulation and potential contamination of the soil with lead (Pb) is shown in Table 11 and 12. The total concentrations of Pb determined in this study were relatively low and remained below the maximum permissible concentration (MPC) of 50 mg Pb kg^{-1}. In both years, no significant differences in soil Pb concentrations were observed between treatments during the trial. As the Pb content in the applied digestate was low, no adverse effects on the soil were expected or observed, as in Przygocka-Cyna and Grzebisz (2018), where the values in the soil ranged between 4.50 and 4.60 during the three year experiment. These values are well within the typical range of Pb concentrations in soils, which largely depend on the parent material and generally range from 2 to 100 mg Pb kg^{-1} (Briseño-Bugarín et al., 2024).

The average Pb content in plants is approximately 2 mg Pb kg^{-1} of dry matter (Kabata-Pendias and Pendias, 2001). In this study, the total Pb content in maize was higher and more variable, depending on growth stage and year. In 2018, Pb concentrations ranged from 2.2 to 6.2 mg Pb kg^{-1} at the V4 stage, 1.4 to 4.6 mg Pb kg^{-1} at the R5 stage, and 1.9 to 4.7 mg Pb kg^{-1} at the R6 stage. In 2019, the corresponding values ranged from 3.1 to 8.3 mg Pb kg^{-1} at the V4 stage, 1.8 to 6.2 mg Pb kg^{-1} at the R5 stage, and 1.9 to 4.7 mg Pb kg^{-1} at the R6 stage. These results indicate considerable variability in Pb concentrations among treatments and growth stages. As with Ni, one possible explanation for this variability is the proximity of the experimental plot to a road, a known source of heavy metal contamination. Importantly, the digestate from the Bojana biogas plant did not contribute to harmful accumulation of Pb in the soil or plant material.

According to Čoga et al. (2019), the average chromium (Cr) concentrations in soil are between 7 and 221 mg Cr kg^{-1}, depending on the soil type and the parent material from which the soil was formed. If the measured concentrations are compared with the maximum permissible concentration (MPC) of 40 mg Cr kg^{-1} (as shown in Table 11 and 12), it can be seen that the initial Cr content of the soil in 2018 before the start of the trial exceeded the MPC by 1.66 mg Cr kg^{-1}. In 2019, this concentration was slightly below the limit at 39.69 mg Cr kg^{-1}. One possible explanation for the increased Cr concentrations in the soil is the proximity of the test site to a road, as the applied digestate had a low Cr content and are probably not the source of the contamination.

The Cr content in plants usually ranges from 0.02 to 0.2 mg Cr kg^{-1} DW (Kabata-Pendias and Pendias, 2001). In accordance with the results, the total Cr concentrations in the plant material followed a uniform trend across all growth stages. According to Smilde (1976), a Cr concentration of 80 mg Cr kg^{-1} had no adverse effects on maize grown on sandy loam soil with a pH of 5.5. Since the soil in this study had an even lower pH, it is likely that Cr assimilation by the plant was further inhibited, which could explain the relatively low accumulation of Cr in plant tissue despite elevated soil levels.

With regard to cobalt (Co), the Ordinance on the Protection of Agricultural Land from Contamination by Pollutants (NN 71/2019) prescribed that the maximum permissible concentration (MPC) of Co in soil is 30 mg Co kg^{-1}. According to Vukadinović and Vukadinović (2011), the average Co content in soils ranges from 0.02 to 0.5 mg Co kg^{-1} of soil. In both years of the study and in all treatments, there were no statistically significant differences in the total Co concentrations in the soil and the values remained below the maximum limit.

The Co content in plants generally ranges from 1 to 40 mg Co kg^{-1} of dry matter (Vukadinović & Vukadinović, 2011), although lower concentrations are more commonly reported. Bakkaus et al. (2005) indicate that typical Co levels in plant tissue are between 0.1 and 10 mg Co kg^{-1}. In our study, Co concentrations in maize remained within this optimal range, varying from 0.23 to 2.09 mg Co kg^{-1} in 2018 and from below the detection limit to 1.35 mg Co kg^{-1} in 2019.

These results indicate that there is no risk of Co accumulation in plant tissue and there is no evidence of cobalt-related contamination from the applied treatments.

Cadmium (Cd) is a highly toxic element for humans, animals and plants, even in very low concentrations. According to Čoga et al. (2019), the average Cd concentration in the lithosphere is around 0.2 mg Cd kg^{-1}, while in soils it is typically between 0.01 and 0.7 mg Cd kg^{-1}.

The influence of digestate application on the Cd concentration in the soil is shown in Table 11 and 12. Across all treatments and sampling periods, the total Cd content remained well below the MPC of 1 mg Cd kg^{-1}, with the highest value being 0.42 mg Cd kg^{-1}. These low values were to be expected, as the digestate used contained less than 1 mg Cd kg^{-1} of dry matter. In a comparable study by Przygocka-Cyna and Grzebisz (2018), Cd concentrations between 0.14 and 0.25 mg Cd kg^{-1} were found over a period of three years after the application of digestate, which were also below the MPC under neutral soil pH conditions.

According to Gjorgieva Ackova (2018), the average Cd) content in plants ranges from 0.2 to 0.8 mg Cd kg^{-1} DW. Cadmium concentrations measured in the aboveground maize mass were less than 1 mg Cd kg^{-1}, which is well below the critical toxicity threshold for most plants (5 mg Cd kg^{-1}). These values are significantly lower and do not pose a phytotoxic risk. Therefore, the risk of Cd transfer to animals or humans through the food chain is negligible (Čoga et al., 2019). Based on these results, the digestate from the Bojana biogas plant is suitable for agricultural use and does not contribute to increased Cd accumulation in maize or potential contamination of the food chain.

5.1.2. Effects of fertilizer use on maize (*Zea mays* L.) production

During the two-year study period, significant differences in fresh and dry biomass yields were observed, which were influenced by the growing season, treatment type and vegetation stage. The application of digestate contributed to the improvement of soil health, fertility and maize productivity. On average, fresh and dry biomass yields peaked in 2019 ($p<0.0001$). The most productive treatments throughout the study were NPK+CAN and the combinations of digestate fractions with mineral fertilizers (SFD+NPK and LFD+NPK). The reproductive stages (R5 and R6) consistently produced significantly higher biomass compared to the vegetative V4 stage in all treatments ($p<0.0001$).

Sigurnjak et al. (2017) recorded fresh biomass yields of 77 t ha^{-1} under mineral fertilizer in combination with pig slurry, with slightly lower values for digestate treatments. In comparison, the present study found average fresh biomass yields of 69.7 t ha^{-1} for NPK+CAN, 64.6 t ha^{-1} for LFD+NPK, 64.1 t ha^{-1} for SFD+NPK, 59.0 t ha^{-1} for LCM, 58.1 t ha^{-1} for LFD, and 56.2 t ha^{-1} for SFD.

Despite higher fresh and dry biomass yields in 2019, the average dry grain yield in 2018 (10.9 t ha^{-1}) was higher than in 2019 (9.6 t ha^{-1}). In both years, the highest grain yields were achieved with the treatments NPK+CAN and LFD+NPK (12.1 and 11.9 t ha^{-1}, respectively).

Fresh biomass yields were the highest in the NPK+CAN treatment and in the digestate-mineral fertilizer mixtures, with similar trends observed in dry biomass production. In contrast, the control treatment (C) consistently had the lowest biomass yields at both the R5 and R6 stages. It is likely that hail damage in June 2018 (Table

2) negatively affected green biomass yields in R5, although favorable weather later in the season supported a partial recovery.

Dry biomass yields were slightly higher in the 2018 at the R5 stage compared to 2019. Across all treatments, dry matter increased by approximately 5.5 % at the R6 stage, likely due to more consistent rainfall in June and August (Table 3). High temperatures and drought stress in these months usually lead to a reduction in biomass and grain yields, especially when nutrient or water availability is limited. The same situation occurred in 2019, when there was drought in August. At the end of the experiment, the yield was lower than in 2018. According to Corteva Agriscience, the average fresh biomass yield for Croatian farmers at R5 is 55.46 t ha^{-1}, while Croatian national statistics from 2013–2017 (Krištof, 2018) show yields between 30.3 and 41.4 t ha^{-1}, which is consistent with the range observed in this study (29.6–37.5 t ha^{-1}).

Precipitation during the growing season had a notable impact on the relationship between biomass production and grain yield. Although dry matter was slightly higher at R5 in 2018, an overall increase of 5.5 % at R6 was attributed to favorable precipitation patterns in June, July and August. The literature indicates that high temperatures in these months combined with limited water availability or low humidity can greatly reduce yields (Pucarić et al.,1997). In addition, drought conditions in August likely contributed to the lower dry matter yields in both study years, especially in 2019.

Although biomass production was statistically higher in 2019, dry maize grain yields were consistently higher in 2018 in all treatments. The treatments with NPK+CAN (12.1 t ha^{-1}) and the LFD+NPK mixture (11.9 t ha^{-1}) consistently achieved the highest yields. Seasonal differences in grain yield can be attributed to climatic conditions, soil properties and pest pressure. In particular, maize rootworm (*Diabrotica virgifera virgifera*), which damages maize silks during the R1 stage, may have negatively affected pollination and grain development, especially under the dry conditions from June to August. This insect damage can lead to incomplete pollination and abortion of grains (Culy et al., 1992; Tollenar and Dwyer, 1999; Westgate et al., 2004; Hrgović, 2007; Doyeni et al., 2021).

When analyzing the individual seasons, the highest dry grain yields were recorded in 2018 for treatments with NPK+CAN (13.1 t ha^{-1}), SFD+NPK (11.2 t ha^{-1}) and LFD+NPK (12.2 t ha^{-1}), while in 2019 the same treatments achieved slightly lower yields: NPK+CAN (11.1 t ha^{-1}), SFD+NPK (10.8 t ha^{-1}), and LFD+NPK (11.5 t ha^{-1}).

The lowest grain yields were observed in the unfertilized control treatment in both years. National data reported by Cvjetićanin et al. (2017) showed that Croatian maize grain yields ranged between 6.5 and 8.5 t ha^{-1} between 2013-2017. In comparison, the average dry grain yields during the two-year study period were 7.9 t ha^{-1} for the control, 12.1 t ha^{-1} for NPK+CAN, 9.9 t ha^{-1} for LCM, 9.4 t ha^{-1} for SFD and 9.6 t ha^{-1} for LFD treatments. These results are comparable to those of Chantigny et al. (2008), where in a three-year study comparing mineral fertilizers with pig manure and digestate, similar dry yields were observed with a total N application of 130 kg N ha^{-1}. The study by Przygocka-Cyna and Grzebisz (2018) reports lower yields than those achieved in this trial. Over three years of digestate application (application rates of 0.8, 1.6 and 3.2 t ha^{-1}), they recorded yields between 8.8 and 10.63 t ha^{-1}.

Regarding maize grain properties, nitrogen (N) directly influences yield, grain protein content (Tsai et al., 1978; Pearson and Jacobs, 1987; Uhart & Andrade, 1995), and grain hardness (Tamago et al., 2016), and also affects zein concentration in the endosperm. Consistent with these established roles, the present study clearly demonstrates that N availability is a key determinant of zein accumulation. Similar effects of N on zein concentration have been reported under both field conditions (Gerde et al., 2016; Tsai et al., 1978; Tsai et al., 1984; Ahmadi et al., 1995) and in vitro experiments (Singletary et al., 1990).

In agreement with these findings, Figure 4 shows that grain N content was strongly and positively correlated ($p < 0.0001$) with total protein (99.93%) and zein concentration (73.91%) across all treatments, whereas starch concentration was negatively correlated with N content. This inverse relationship may be partly explained by environmental conditions during the growing season. From June to August 2018, drought stress likely contributed to reduced starch accumulation, while heat stress is known to decrease starch deposition and increase protein concentration in maize grains (Tester and Karkalas, 2001; Wang & Frei, 2011; Thitisaksakul et al., 2012), a pattern also observed in the present study (Šatvar Vrbančić et al., 2025).

This study highlights the central role of N accumulation in maize grains, particularly in the form of storage proteins, which are strongly correlated with zein and total protein content. The field experiment confirmed that grain N content was closely associated with protein and zein concentrations, and that the highest grain yields were achieved under mineral fertilization (NPK+CAN) and combined treatments (SFD+NPK and LFD+NPK). These results underscore the synergistic benefits of integrating digestate with mineral fertilizers to enhance both grain yield and quality.

5.1.3. Nutrient uptake by the maize

The soil pH (Table 5) probably influenced the nutrient uptake of maize, especially by immobilizing certain elements (Figure 33). The critical soil pH range for optimal maize growth is reported to be between 5.0 and 5.5 (Rhoads & Manning, 1989) or between 5.51 and 7.2 (Mandić et al., 2023; Herak Ćustić et al., 2025). However, the soil pH of the field in this study was even lower, as shown in Table 5. Considering the very acidic conditions, there was an increased risk of reduced nutrient uptake, mainly due to reduced microbiological activity. In such soils, the uptake of macronutrients and molybdenum tends to decrease, while the solubility and availability of several micronutrients increases. When mineral fertilizers are applied, ammonium N is quickly oxidized and converted into nitrate, while processes such as proteolysis, decomposition, nitrification and ammonification must first take place in organic materials such as fermentation residues (Herak Ćustić et al., 2025). Above all, in the beginning of the experiment digestate can help to increase the pH value of the soil, as already explained, which has positively effected on nutrient uptake (nutrients) as well as improving microbiological activity and general soil health.

It is well known that the uptake of Ca and Mg decreases in all plant species when the soil pH decreases (Alam, 1999), while the uptake of micronutrients such as Zn, Mn and Fe tends to increase under more acidic conditions. However, the effects on macronutrients are species specific, for example, a higher soil pH tends to reduce the uptake of N, P and K in rice, while it improves uptake in maize, wheat and field beans (Frageria and Zimmermanna, 1998).

The results of this study showed that the uptake of N, P, Fe, Zn and Cu was significantly higher in 2018 than in 2019 ($p<0.0001$), while the uptake of Ca, Mg and Mn was higher in 2019 ($p<0.0001$). The weather conditions between the two growing seasons played an important role in these differences. In May 2018, the average temperature was 19.5 °C, while in May 2019 it was 13.7 °C, a difference of 5.8 °C. The higher temperature in 2018 probably accelerated the mineralization of organic matter and the release of N, which improved the availability of P, Fe, Zn and Cu. In addition, a total of 127.8 mm of precipitation fell in June 2018, about 50 mm more than in June 2019, which further supported nutrient uptake this year.

Conversely, the higher total precipitation in 2019 favored the uptake of Ca and Mg, which mainly move through the soil via mass flow (Bergmann, 1992). No significant differences were found between the two seasons with regard to K uptake.

Analyzing the percentage change in metal uptake between 2018 and 2019, Ca uptake increased by 43±19 % on average across all treatments, while Mg uptake increased by 24±9 %. On the other hand, Zn uptake fell by an average of 14±6 % and Cu uptake fell sharply by 42±12 %. Drought stress in 2019 probably contributed to the reduced Cu and Zn uptake, as water scarcity limits microbial activity, which plays a crucial role in nutrient availability (Schimel, 2018).

It has also been reported that the use of bio-based fertilizers can lower soil pH, increasing the availability of Fe and inorganic N, a trend that was also observed in this experiment (Ali et al., 2015). In fact, Fe uptake increased by an average of 27±11 % between 2018 and 2019 across all treatments. As Marschner (2012) noted, soil properties largely determine how plants respond to nutrient availability, with soil pH being a key factor in nutrient uptake. In this study, the application of digestate helped to improve the physico-chemical properties of the highly acidic soil, increase nutrient availability and slightly reduce soil acidity over the course of repeated applications over two years, as in Doyeni et al. (2021) and Glowacka et al. (2020) studies.

5.1.4. Apparent Nitrogen Recovery (ANR) and Nitrogen Fertilizer Replacement Value (NFRV)

As Cavalli et al. (2016) pointed out, the efficiency of N uptake from untreated manure and anaerobic by-products (digestate) is usually assessed by calculating the Apparent Nitrogen Recovery (ANR) and Nitrogen Fertilizer Replacement Value (NFRV). In this study, the lower ANR and NFRV values observed in all bio-based treatments likely resulted from a lower initial NH_4-N/total N ratio in the digestate products tested (Reijs et al., 2007), especially in 2018 (Table 15). The ratio of NH_4-N to total N was statistically higher in 2019 ($p<0.0001$) than in 2018, which can be attributed to changes in the composition of the feedstock in the biogas plant, in particular an increased proportion of LCM added in 2019. As a result, the NFRV values increased slightly in 2019 for all treatments, but the differences were not statistically significant.

In 2018, the highest ANR values were recorded in the treatment with NPK+CAN and LFD+NPK (Table 15). Similarly, these two treatments also achieved the highest NFRV values among all treatments. A similar pattern was observed in 2019, with the highest ANR values recorded for the NPK+CAN, SFD+NPK and LFD+NPK treatments. Statistically, NPK+CAN and the LFD+NPK combination consistently provided the highest NFRV values. After the NPK+CAN treatment (100±0), the LFD+NPK treatment had the second highest NFRV at 83±9. However, over the two years of the trial, the NFRV for the LFD+NPK treatment decreased by approximately 6%.

The relatively lower ANR and NFRV values observed in the LFD and SFD treatments could be influenced by several factors, including differences in the carbon-to-nitrogen (C:N) ratio. A higher C:N ratio may lead to nitrogen immobilization or delay the mineralization of organic N, especially in the first years after application. Additional N losses may have resulted from the volatilization of ammonia, especially given the speed of incorporating fertilizers into the soil and acidic soil conditions (Sommer and Hutchings, 2001; Cavalli et al., 2016).

As mentioned in the introduction, the NH_4-N/total N ratio in digestate is directly influenced by the type of feedstock used in AD, which ultimately affects the NFRV.

According to Dai and Karring (2014), pig manure generally contains higher levels of N, both organic and ammoniacal than cattle manure, which explains the typically higher NFRV of pig manure. In agreement with this, Sigurnjak et al. (2017) reported NFRV values of over 90% when using pig manure, a figure slightly higher than the results obtained in the present study.

Comparative studies by Cavalli et al. (2016) showed that using cattle manure, NFRV values in the LFD increased from 25–30 % in the second year to 75–80 % in the third year of application, while values for the solid fraction of the digestate (SFD) increased only slightly from 20 to 25 % over the same period. In contrast, our investigations showed that the NFRV values for LFD (48±2%) and SFD (31±6%) were already higher in the second year.

From a practical point of view, especially in the framework of sustainable agriculture, NFRV is a key indicator as it reflects the efficiency of nitrogen use, contributes to better crop yields, reduces dependence on mineral fertilizers and minimizes environmental impacts.

5.1.5. Post-harvest soil properties

Nitrogen that is not taken up by the plant is prone to ammonia volatilization, denitrification and leaching, leading to environmental problems and NO_3^--N leaching (Sigurnjak et al., 2017; De Vos et al., 2000). The top layer of the soil (0-25 cm) is most affected by N fertilization according to Chen et al. (2016); Janzen et al. (1990) and Zilio et al. (2020).

Soil NO_3^--N residues were sampled (0-90 cm) during post-harvest sampling in 2018 and 2019 to determine the concentration and potential risk of NO_3^--N leaching. The experimental field soil contains 15.4% sand, 67.5% silt and 17.1% clay, and its texture is categorized as silt loam soil. According to Vukadinović and Vukadinović (2000), soils can be classified into at least five classes: from very light texture to very heavy texture. Experimental soil belongs to the medium-heavy soil class, what means that it must be plowed and tilled/cultivated at the right time to reduce the loss of plant nutrients through leaching.

In 2018, statistical differences were found between the treatments where NPK+CAN and a mixture of LFD+NPK resulted in the highest NO_3^--N residues in the soil after harvest. The increased risk of nitrate leaching in 2018 was due to the improved conditions for mineralization, which significantly enhanced the processes of ammonification and nitrification. The precipitation in June 2018, which amounted to 127.8 mm, contributed to increased nitrate leaching. In addition, the favorable temperatures in May and June promoted better mineralization and nitrification. In the treatments fertilized with NPK+CAN and in the mixtures of NPK with digestate fractions, a higher proportion of total mineral N was present in the soil in 2018 than in 2019. Consequently, these treatments (NPK+CAN and LFD+NPK) had a positive effect on the soil and led to a higher content of mineral N. During second year of experiment (2019), there was no significant difference between the treatments, but the highest NO_3^--N residues were recorded in the treatments where NPK+CAN was used. In 2019, post-harvest soil NO_3^--N residue concentrations were lower. In the Zilio et al. (2021) two-year study, soil nitrate content was mainly affected in the top soil layer (0–25 cm), where a significant increase was observed after fertilization with 41.82 ± 11.6 mg kg^{-1} for synthetic fertilizer (SF) and 45.57 ± 6.81 mg kg^{-1} for recovered fertilizer (RF) treatments. Deeper soil layers (25–100 cm) had minimal nitrate levels, indicating that nitrate leaching was limited even in RF plots that received almost double the N dose (460 kg N ha^{-1}) compared to SF while in our study 140 kg N ha-1 was used as a fertilization rate. Likewise, Sigurnjak et al. (2017) in their 3-year study resulted in higer NO_3^--N in treatment liquid fraction of digestate+digestate (LFDIG+DIG) as compared to reference treatmen synthetic fertilizer+animal manure (SF+AM). Eventhought the NO_3^--N levels were high, they were still below the legislative maximum allowable level of 80 kg NO3--N ha-1 (0-90 cm) for Flemish soils in both 2012 and 2013. Interestingly, back in 2011, all treatments including the reference, treatment exceeded that limit, suggesting that factors other than fertilizer type may have contributed to nitrate buildup that year. As well, this is in line with the results of Zilio et al. (2021), which also show that despite the higher total N input in RF plots, NO3--N levels at harvest (e.g. 0.41 ± 0.12 mg kg^{-1} DW in 2019) were lower or comparable to unfertilized plots, suggesting efficient plant uptake and/or transformation processes to mitigate environmental risk.

The results indicate that the use of SFD and LFD compared to NPK+CAN does not increase the risk of nitrate residues or leaching, which is consistent with our third hypothesis that digestate fertilization has no harmful effects on the soil or plant through increased nitrate leaching. Additionally, the studies show no negative impact on the environment, as the negligible amounts of leached nitrates at a depth of 0-90 cm are insignificant. In soils with low organic matter and low pH, the application of organic material such as digestate has a positive effect on the physical and chemical properties of the soil by increasing the amount of organic matter, enhancing microbiological activity and improving the nutritional value by adding important nutrients (Šatvar Vrbančić et al., 2024).

The concentration of NO_3^--N residues in the soil was further followed by treatment with a mixture of SFD+NPK and LCM. As can be seen in Figure 20 the NPK+CAN treatment had the highest NO_3^--N residues in the soil after harvest. In 2019, there was no significant difference between the treatments, but the highest NO_3^--N residue was recorded in the NPK+CAN treatment. The year 2019 showed lower concentrations of NO_3^--N residues in the soil after harvest. The results indicate that the use of SFD and LFD should not additionally increase the risk of nitrate residues or leaching compared to NPK+CAN.

According to Havlin et al. (2014), ammonia in the soil is strongly influenced by fertilizer form, microbial activity, and environmental conditions, particularly temperature and soil moisture. $NH4^+$–N is typically present at lower and more transient concentrations than nitrate because it is rapidly oxidized to NO_3^- through nitrification under aerobic conditions. This explains the pronounced early-season $NH4^+$–N peaks observed in mineral fertilizer treatments such as NPK+CAN, SFD+NPK, and LFD+NPK, followed by a rapid decline later in the season. Similar temporal patterns were reported by Cui et al. (2010) and Diacono and Montemurro (2010), who showed that ammonium supplied by mineral or readily mineralizable organic fertilizers is quickly transformed or taken up by plants during periods of active growth. In contrast, the consistently low $NH4^+$–N levels in the control and purely organic treatments suggest slower N mineralization and greater microbial immobilization, reducing the risk of ammonium accumulation. Seasonal overlap of $NH4^+$–N concentrations across treatments toward harvest further indicates efficient plant uptake and nitrification, particularly under favorable soil aeration. Differences between years, with generally higher $NH4^+$–N residues in 2018 than in 2019, are likely due to variations in temperature and precipitation influencing mineralization and nitrification rates (Norton & Stark, 2011). Overall, the observed ammonium dynamics support the conclusion that mineral N availability was highest early in the growing season, with no evidence of harmful $NH4^+$ accumulation under any fertilizer treatment.

As a member of the EU-27, Croatia is subject to the Regulation on the maximum permitted levels of nitrates in groundwater and surface waters, which is 50 mg L-1. As mentioned above, the Nitrates Directive (91/676/EEC) prescribes 170 kg N ha-1 as the maximum dose for total N fertilization on nitrate voulnerable zone (NVZ). In the EU-27, the NVZ are quite heterogeneous from area to area. Some Member States have decided to ensure the same level of protection throughout their territory, while others have established NVZ (NN 60/2017). According to Ondrašek et al. (2021), the current situation in Croatia shows that nitrate vulnerable zones (NVZ) cover less than 10 % of the national territory, and total soil mineral N (TSMN) dynamics were almost the same in both years at the beginning of the experiment. In 2018, the TSMN was 37±3 kg ha-1, while in 2019 it was 38±2 kg ha-1. At the beginning of the experiment, the previous crop on the experimental field was soybean. According to Vratarić and Sudarić (2000), soybean enriches the soil with 40-60 kg N ha-1, which corresponds to the amount of N that was present before the start of the trial. As shown in Figure 21, a mixture of LFD+NPK treatment had the highest TSMN content at vegetative stage V4 in both years, while at reproductive stage R5, NPK+CAN treatment had the highest TSMN content of all treatments in both years. After fertilization, the TSMN concentration increased from pre-sowing to reproductive stage R5 in all treatments and then decreased until reproductive stage R6 in all treatments in 2018 and 2019 (plant uptake). Since the dry period from June to August, N uptake has been lower (the elements are unavailable due to the low precipitations and high temperatures). After the 2018 harvest, some of the N remained in the soil and since the same experiment was set up the next year, the 2018 crop residues were ploughed under and mineralized over time.

This was also detected when the soil was analysed before the new experiment. A certain amount of TSMN was present in the soil before the 2019 experiment.

Soil nitrogen content was monitored in the study by Zillio et al. (2023) during the 2019 and 2020 maize growing seasons by analyzing nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations across soil depths. The surface layer (0–25 cm), most responsive to fertilization, showed the greatest N fluctuations.

5.1.6. Mass balance and environmental relevance of heavy metals

A mass balance approach was used throughout the two-year experiment to determine the potential for heavy metals to accumulate in the soil. The balance for each element was calculated as the total amount of fertilizer applied minus the total amount removed in harvested crops. These balances were then converted into changes in soil concentration (mg kg^{-1}), assuming a soil depth of 0–30 cm and a bulk density of 1.4 g cm^{-3}, which equates to approximately 4.2×10^6 kg soil ha^{-1}.

The calculated changes in heavy metal concentrations in the soil were very small, typically only a few mg kg^{-1}. These values are much lower than the natural spatial variability usually observed in agricultural soils and fall within the range of error for routine soil measurements. Similar results have been reported in nutrient budgeting and other field studies, indicating that minor positive element balances do not necessarily result in measurable increases in soil concentrations (Oenema et al., 2003; Salo et al., 2006).

Although there were statistically significant differences between treatments, the magnitude of the calculated changes indicates that short-term accumulation is negligible. When assessing the risk of heavy metals, it is important to consider how the added elements mix with the existing soil and background concentrations (Nicholson et al., 2003; Nkoa, 2014). When elements are distributed across several million kilograms of soil per hectare, even small additions measured in kg ha^{-1} have little effect on overall soil concentration.

5.1.7. Nutrient balances and soil processes

Nutrient balances are an important indicator of soil fertility, as they show whether nutrient reserves are being depleted, maintained, or improved. This, in turn, affects the long-term productivity of the soil. The balance between nutrient inputs and outputs in the field is often assessed to track changes in agricultural systems. When crops remove more nutrients than they add, a negative nutrient balance occurs, leading to soil fertility depletion or nutrient mining. Conversely, positive nutrient balances show that nutrients are accumulating and that soil fertility may improve (FAO, 2006; FAO, 2025).

Notably, the nutrient balance was negative across all treatments, indicating that crop uptake exceeded fertilizer input. This suggests net depletion of plant available N, which may be partly compensated by mineralization of soil organic matter. In acidic silt loam soils, nitrogen dynamics are strongly influenced by mineralization and immobilization processes and potential nitrate leaching (Zhang et al., 2019). Given the soil texture and acidity, it is possible for nitrate to be lost through leaching, especially under mineral fertilization, which may explain persistent negative balances even in fertilized treatments.

Phosphorus balance showed both positive and negative values depending on treatment. In acidic soils, P availability is strongly controlled by sorption to iron and aluminium oxides (Hinsinger, 2001). Thus, positive P balances do not necessarily imply immediate plant availability or environmental risk, as a portion may be immobilized in less soluble forms. On the other hand, negative balances may reflect removal exceeding available input but do not necessarily indicate rapid depletion of total soil P reserves.

Potassium balance remained negative in all treatments, suggesting ongoing K removal. In silt loam soils, K dynamics depend on exchangeable and non-exchangeable pools, with partial buffering capacity (Zörb et al., 2014). Continued negative balances may gradually reduce exchangeable K if not compensated.

Magnesium and calcium balances varied among treatments. The positive Ca balance observed in digestate treatments is consistent with the liming effect often reported for digestate derived amendments (Möller & Müller, 2012). In acidic soils, Ca inputs may partially neutralize acidity and influence nutrient availability and metal mobility.

5.1.8. Heavy metal behavior in acidic silt loam soil

Soil pH is an important factor influencing the mobility of heavy metals. In acidic soils, metals such as Zn, Cu, Ni, and Cd are generally more soluble and potentially more mobile (Alloway, 2013). However, increased mobility does not necessarily result in accumulation. In this study, negative mass balances were frequently observed for Ni, Pb, and Cd, indicating that the amount removed by crops was equal to or greater than the amount added.

Recent research has extensively examined the use of digestate, with several studies reporting negligible short-term accumulation of heavy metals when application rates comply with regulatory standards (Nkoa, 2014; Insam et al., 2015; Tambone et al., 2017). Long-term experiments show that measurable increases in soil heavy metals typically occur only after decades of repeated application at much higher loading rates (Nicholson et al., 2003).

In the present study, even treatments with positive mass balances showed theoretical increases that were too small to exceed analytical detection limits. This supports recent meta-analyses demonstrating that short-term digestate application does not pose a significant contamination risk when initial metal concentrations are low and application rates are agronomically appropriate (Salo et al., 2006; Möller & Müller, 2012).

Eventually, ANOVA indicated statistically significant differences between treatments for several elements. However, these differences were minor. Statistical significance here reflects minimal variability among treatments rather than substantial environmental changes. When considered in the context of expected variations in soil concentration, the differences among treatments fall within the range of natural soil heterogeneity and measurement uncertainty.

This suggests that soil analytical data alone, without contextual mass balance evaluation, may over emphasise small changes that are environmentally insignificant. The mass balance approach provides a more accurate assessment of contamination risk and demonstrates that the materials used do not pose a measurable short-term threat to soil quality.

5.2. Laboratory experiment

To put our results in perspective, we compared them with the results of other similar incubation studies in which nitrogen (N) mineralization from both mineral and organic sources was investigated. Sigurnjak et al. (2017) conducted a 120-day experiment comparable to ours.

Sigurnjak et al. (2017) applied 150 kg N ha^{-1} and used calcium ammonium nitrate (CAN) as their reference material, just like in our setup where we use mixture of NPK+CAN as a reference material. In their study, the CAN treatment showed a complete N release, with a net N mineralization ($N_{rel,net}$) of 103 ± 4 %. This value is slightly higher than the 87 ± 5 % we observed in our own NPK+CAN referent treatment, but is still in a comparable range, underlining the predictable efficiency of mineral fertilizers and their fast release.

Reuland et al. (2022), who applied the same amount of N as we did (170 kg N ha^{-1}), evaluated a range of different organic sources (digestate from biowaste, from sewage sludge, from maize silage, from pig manure, from chicken manure and reference materials, commercial compost, undigested pig slurry and solid fraction of digestate from chicken manure). Their results showed high mineralization rates for undigested pig manure (87 %), digestate from chicken manure (81 %) and digestate from pig manure (78 %). Even the solid fraction of digestate derived from chicken manure achieved a mineralization of 48 %. In contrast, our solid fraction of digestate (SFD) digested from cattle manure yielded a much lower $N_{rel,net}$ of only 13 %. This clear difference likely comes from the naturally lower ammonia content of cattle manure compared to poultry manure, which tends to contain a higher proportion of readily available nitrogen (Beauchamp, 1986; Vukadinović & Vukadinović, 2011). These differences in feedstock composition and treatment history highlight the importance of understanding the origin of organic materials when predicting their behavior in soil.

Luo et al. (2021) compared calcium ammonium nitrate (CAN), pig slurry (PS) and the liquid fraction of digestate (LFD) at 150 kg N ha^{-1} in soil-only incubations and in maize-growing pots. On day 20, CAN showed net N immobilization (−14±7%), but mineral N release increased to 106 ± 1% by day 80, while at day 20 in our study immobilization of NPK+CAN also occurred (-26±2%), while we had increase to 90±3% at day 60, and at day 80 again immobilization (-13±3%). PS and LFD maintained positive net N release, reaching 84.4 ± 5.5% and 99.0 ± 2.9%, respectively, same as in our treatments with LFD+NPK and LFD. At day 100, the release decreased to 96.9, 68.5 and 78.8% for CAN, PS and LFD, respectively, probably due to the increased N content in the control. If compared to LFD only, it increased to 50% by day 120. LFD performed better than PS in the Luo et al. (2021) incubations, same as LFD was better tham LCM in our study.

Tambone and Adani (2017), who applied a higher N rate of 300 kg N ha^{-1} and conducted a slightly shorter 90-day incubation, used urea as a reference treatment and also tested a range of organic materials, including digestate, sewage sludge, compost and a control (unfertilized). They observed a sharp decrease in NH_4^+-N content in digestate-treated soils shortly after application. This initial decrease was similar to that observed in our study, although in our case the decrease in ammonium was generally less pronounced in the organic treatments. Notably, all three studies - ours, Tambone and Adani (2017), and Sigurnjak et al. (2017) showed a common trend: an initial decrease in NH_4^+-N followed by a short-term increase, especially in the mineral fertilizer treatments. This reflects the hydrolysis of urea and subsequent release of NH_4^+ from sources such as CAN, a pattern that was also clearly observed in our NPK+CAN referent treatment.

An important and consistent difference between the studies was the influence of soil pH on N transformation processes, especially nitrification. Both Sigurnjak et al. (2017) and Tambone and Adani (2017) conducted their experiments in soils with pH values closer to the optimal range for nitrification (6.6 to 8.0), which likely supported a more efficient conversion of ammonium to nitrate. In contrast, our soil reacted strongly acidic, with a pH_{KCl} of 3.93 ± 0.11. This low pH likely suppressed the microbial activity responsible for nitrification, which would explain the slower accumulation of NO_3^--N observed in our study. This limitation is clearly visible in our results (Figure 1), where nitrate levels remained lower than those observed in more neutral soils.

Despite the challenges posed by the acidic soil, we observed immediate N mineralization in the treatments containing the liquid fraction of the digestate (LFD) and LFD+NPK, possibly related to the relatively high ratio of ammonium over total N ratio indicating a more labile form of N that microbes can quickly access. In contrast, the SFD and LCM treatments showed initial N immobilization that delayed mineralization. This pattern is consistent with the early immobilization phases observed by Sigurnjak et al. (2017) for various organic materials and suggests that not all organic fertilizers are equally suitable for immediate nutrient release.

Another notable observation is the timing of highest peak mineralization. In the study by Sigurnjak et al. (2017) the highest $N_{rel,net}$ values were reached around day 80, indicating a more gradual release of N. In our case, all fertilized treatments reached their maximum mineralization on day 60, indicating a faster release of available N. Although the peak was reached earlier, our total $N_{rel,net}$ values were slightly lower at the end of the incubation period. This could be due to either a smaller pool of mineralizable N in our materials or an earlier slowdown in microbial activity.

In all mentioned studies, the control treatment (unfertilized) consistently had the lowest NO_3^--N levels, while mineral treatments such as CAN, NPK+CAN and urea - gave the highest values highlighting their predictable and rapid availability of N. Overall, our results agree well with previously reported findings and confirm that mineral fertilizers provide an immediate and consistent release of N while organic materials show a much more variable behavior. Their performance is strongly influenced by source material, treatment processes, and soil characteristics - particularly pH. These comparisons underline the importance of considering not only the total N content of fertilizers, but also their form, origin and interaction with soil conditions. This is especially important when selecting organic fertilizers for acidic soils, where the timing and efficiency of N release can vary greatly.

Therefore, both short- and long-term N dynamics (mineralization and release) should be carefully evaluated to optimize fertilization strategies and improve nutrient use efficiency in sustainable agricultural systems. Additionally, these observations are in a line with our hypothesis that mineralization and release of N from digestate will increase with time interval from treatment.

5.3. Laboratory and field comparison

The differences between laboratory and field results observed in this study closely follows patterns reported in previous research comparing N mineralization under controlled (laboratory) and field conditions. In the laboratory experiment, NH_4^+ accumulated consistently throughout the incubation, while nitrification progressed more slowly. This response is expected, as the constant air temperature of 18 °C and stable soil moisture (50% WFPS) created optimal conditions for organic N mineralization but did not allow for NO_3^- losses or external environmental disturbances (precipitations, air temperature fluctuations, plant root uptake, microbial immobilization, ect.). Similar behavior has been widely described in incubation studies, where stable conditions favor net mineralization and the buildup of inorganic N, particularly NH_4^+ reflecting the soil's potential mineralization capacity rather than its realized N availability (Sistani et al., 2008; Mgolozeli et al., 2024; Cugnon et al., 2024).

In contrast, N dynamics in the field were markedly different. NH_4^+ concentrations declined rapidly, NO_3^- increased sharply, and overall inorganic N levels remained lower than in the laboratory despite ongoing mineralization. These differences reflect the influence of environmental variability, including precipitation, air temperature fluctuations, microbial immobilization, and denitrification, all of which reduce the amount of detectable mineral N under field conditions. Similar differences between laboratory incubations and field measurements have been reported previously and are consistently attributed to these interacting processes (Sistani et al., 2008; Mgolozeli et al., 2024; Cugnon et al., 2024; Clément et al., 2025; Santiago et al., 2025). Together, these findings confirm the view that laboratory incubations primarily quantify potentially mineralizable N, whereas field measurements capture the realized N supply under variable environmental constraints.

The stronger NH_4^+−NO_3^- correlations observed in the field further support this interpretation. Under natural conditions, mineralized NH_4^+ is rapidly nitrified, and nitrate becomes the dominant mineral N form due to its high mobility in soil (Robertson & Vitousek, 2009; Beeckman et al., 2018; Chen et al., 2024). Once formed, NO_3^- readily moves downward through the soil profile with percolating water, explaining why deeper field layers (30−60 and 60−90 cm) in this study showed stronger correspondence with nitrate patterns than with ammonium. This leaching driven redistribution of nitrate is entirely absent from the laboratory's confined 0−30 cm system. Substantial nitrate leaching under agricultural management has been documented across a wide range of systems, with reported losses of 5–26% of applied fertilizer N below the root zone, while improved management strategies can reduce these losses by up to 95% (Riley et al., 2001). More recent syntheses confirm that nitrate leaching remains one of the dominant pathways of N loss from croplands globally (Liu et al., 2024; Hu et al., 2024; Wang et al., 2025). The strong depth related nitrate patterns observed here mirror these findings and highlight environmental processes that cannot be simulated under laboratory conditions.

In addition to leaching, methodological differences also contribute to the weaker correspondence between laboratory and field NH_4^+ values. Soil incubation methods often produce higher mineralization estimates than in situ measurements, particularly when soils are disturbed, aerated, or protected from natural losses. Earlier work demonstrated that conventional core incubations can overestimate mineralization by up to threefold compared with field based or resin core techniques, largely due to increased aeration and the absence of denitrification and drainage losses (Hatch et al., 2000). More recent evaluations similarly conclude that aerobic or anaerobic incubations should be interpreted as indicators of potentially mineralizable N and used in combination with field measurements, modeling, or plant uptake data to assess actual N availability (Cugnon et al., 2024; Dodor et al., 2024; Beikűfner et al., 2025). This distinction closely parallels our findings. Therefore, in the laboratory, NH_4^+ accumulated due to optimal microbial conditions and the lack of nitrate export pathways, whereas in the field $NH4^+$ was rapidly transformed, nitrified, leached, or immobilized.

Within this broader context, air temperature played only a limited role in explaining mineral N variability under field conditions. Regression analyses showed weak and statistically non significant relationships between air temperature and both NH_4^+ and NO_3^- concentrations, indicating that air temperature alone showed limited control over N dynamics within the observed range. Although slightly higher air temperatures may enhance microbial activity and promote nitrification, this effect was insufficient to produce measurable changes in mineral N concentrations. Similar findings have been reported in recent studies showing that air temperature effects on soil N transformations are often secondary to soil moisture, aeration, and substrate availability under field conditions where multiple processes operate simultaneously (Butterbach-Bahl et al., 2016; Liu et al., 2017).

The absence of a strong air temperature signal for nitrate further supports the view that NO_3^- dynamics in open soil systems are primarily determined by leaching, plant uptake, and microbial immobilization rather than by moderate air temperature fluctuations. Because nitrate is highly mobile, its concentration is strongly influenced by water fluxes through the soil profile, and several studies have demonstrated that precipitation patterns and soil moisture override air temperature effects in regulating nitrate accumulation and loss in agricultural systems (Thapa et al., 2016; Petersen et al., 2018). This is consistent with our observation that nitrate concentrations were probably more strongly associated with soil depth and field conditions than with air temperature.

These field based findings are supported by the laboratory experiment, where air temperature was held constant at 18 °C throughout the incubation. Despite the lack of thermal variation, N mineralization and nitrification proceeded efficiently, resulting in continued NH_4^+ accumulation. This confirms that mineral N production can occur under stable thermal conditions and highlights that air temperature primarily sets a permissive background for microbial processes rather than acting as a dominant controlling factor within moderate ranges. Recent incubation studies similarly demonstrate that once optimal air temperature thresholds are met, further increases in temperature do not necessarily translate into higher mineral N availability unless accompanied by changes in moisture or substrate supply (Rousk et al., 2018).

Additionally, a strong correlation between Nmin released during laboratory incubation and N availability under field conditions aligns with recent findings. Laboratory incubations are widely used to estimate potential N mineralization and account for much of the variability in field N dynamics across treatments. However, they often differ in magnitude from field conditions due to environmental complexity not replicated in controlled settings (Lazicki et al., 2019). Research indicates that incubation methods consistently detect relative variations in mineralization among fertilizers and organic amendments. However, actual N release in the field may be influenced by additional factors (climatic conditions), such as prolonged mineralisation, microbial activity, and inputs from deeper soil layers (Pinto et al., 2020). Recent research on bio-based and organic amendments highlights the significant variability in N mineralization dynamics across different materials. It also shows that combined organic-mineral inputs often result in greater N release than organic sources alone, emphasising the need to consider amendment type in field predictions (Agostini et al., 2024; Mgolozeli et al., 2024). These studies collectively support the interpretation that laboratory incubation accurately reflects treatment variations in Nmin release, yet may underestimate total N availability observed in the field, especially when deeper soil processes and ongoing mineralization are noticeable.

Taken together, the correlation and regression results clearly indicate that N form and soil depth were far more important determinants of N dynamics than air temperature. The laboratory experiment represents the controlled potential for N release under idealized conditions, while the field experiment reflects the ecologically realistic fate of mineralized N under fluctuating moisture regimes, active nitrification, and vertical redistribution through the soil profile. Similar conclusions have been drawn in recent comparative studies emphasizing that laboratory incubations provide valuable functional insight but cannot fully reproduce the complexity of field N cycling (Luo et al., 2019; Xu et al., 2020). Overall, the present findings support integrating controlled laboratory incubations and field observations as a robust approach to evaluate soil N behavior and its implications for N management in agricultural systems.

6. CONCLUSION

Based on the results of the study on the utilization of N from digestate as a substitute for mineral fertilizers in two consecutive vegetation years in maize crops and the analysis of macro- and microelements with heavey metals in soil and plant material as well as the degree of contamination within the soil-plant system, the following conclusions can be drawn:

I. The research hypothesis that the combination of mineral fertilizers with the liquid fraction of digestate (LFD+NPK) achieves the best results in terms of maize yield and quality, while allowing the partial replacement of N from mineral fertilizers, has been confirmed.

Although in 2018 the highest maize yield was obtained in the treatment with mineral fertilizer alone (NPK+CAN), it was not statistically significantly different from the treatment in which mineral fertilizer was combined with the liquid fraction of digestate (LFD+NPK). In 2019, however, the highest yield was achieved with the LFD+NPK treatment. Looking at both years together, no statistically significant difference was found between the two treatments, indicating that the LFD+NPK treatment can serve as a partial substitute for mineral fertilizer.

Along with yield performance, maize grain quality was greatly affected by the amount of N available. This was because N levels were positively associated with protein and zein levels and negatively associated with starch levels. These findings demonstrate that the simultaneous application of LFD+NPK maintains high yields and enhances beneficial grain quality traits through improved N nutrition.

II. The research hypothesis that mineralization and N release from digestate would increase over time was confirmed.

The results of the laboratory incubation showed a gradual release of N, which is characteristic of organic fertilizers, while nutrients from mineral fertilizers are immediately available. These results are consistent with previous studies and confirm that organic fertilizers release different amounts of N depending on the source material, treatment process and soil properties, especially pH of the soil. This underlines the need to consider not only the total N content, but also the form of the fertilizer and its interaction with soil conditions, especially in acidic soils. Therefore, both short and long term N dynamics should be evaluated when optimizing fertilization strategies, supporting our hypothesis that N mineralization from digestate increases with time.

The gradual N release observed during incubation is consistent with the field nutrient balance results, where digestate-based treatments showed comparable nitrogen dynamics to mineral fertilization, indicating that digestate can provide a stable supply of N for crop uptake.

III. The research hypothesis that fertilization with digestate has no harmful effects on the pollution of soils and plants with nitrates and heavy metals was also confirmed.

The results showed that the use of both the solid (SFD) and liquid (LFD) fractions of digestate did not increase the risk of nitrate residues or leaching compared to mineral fertilizer treatments (NPK+CAN). This supports the hypothesis that digestate fertilization has no negative effects on soil or plant quality due to increased nitrate leaching. Furthermore, the negligible amounts of nitrate leached at a depth of 0–90 cm suggest that there is no significant impact on the environment.

In addition, results showed no negative effect of the digestate to heavy metal contamination in the plant or soil. Furthermore, the results showed no negative effects of digestate application on the heavy metal load in the plant or in the soil. The heavy metal concentrations remained within acceptable limits and did not differ significantly from those observed in mineral fertilizers treatments. This indicates that the use of digestate does not pose an environmental risk related to the accumulation of heavy metals, supporting their safe use as an alternative fertilizer in sustainable agriculture. In line with this, these conclusions align with the mass balance analysis, which showed that nutrient dynamics remained stable and heavy metal concentrations did not increase, further supporting the safe use of digestate in sustainable fertilization strategies.

7. LITERATURE

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BIOGRAPHY OF THE AUTHOR

Mihaela Šatvar Vrbančić was born on March 4, 1991 in Koprivnica, Croatia. She attended the "Ljudevit Modec" elementary school from 1998 to 2006 and then the "Ivan Zakmardija Dijankovečki" High School in Križevci from 2006 to 2010. She completed her undergraduate studies at the University of Applied Sciences in Križevci, specialising in horticulture, and her postgraduate studies at the Faculty of Agriculture at the University of Zagreb, specialising in phytomedicine. Since 2017, she has been working as an assistant in the Department of Plant Nutrition and is a collaborator in five undergraduate (BS) modules and four graduate (MS) modules. She is also a PhD student in Agricultural Sciences at the Faculty of Agriculture of the University of Zagreb and the joint PhD student at the Faculty of Life Sciences and Engineering of the University of Ghent, Belgium. She won the first prize at the VIII Review of Scientific Papers of Agronomy Students at the Faculty of Agriculture in Čačak, Serbia, during her BS studies in 2013, also won two "Dean's Awards" at the Faculty of Agriculture of the University of Zagreb in 2014 and 2015 and the "Milan Maceljski Foundation" Award in 2015 during her MS studies. From April 1 to July 1, 2013, she participated in the student exchange programme "Erasmus" and completed a professional internship at the Faculty of Agriculture and Biosystems Science in Maribor. During her MS studies, she participated in 2 projects under the supervision of prof. Ph.D. Renata Bažok "Improving sugar beet production technology in accordance with the principles of integrated pest management", funded by the Croatian Science Foundation (2014-2015), and "Improving cooperation between science, industry and farmers: technology transfer for integrated pest management (IPM) in sugar beet as a means to increase farmers' income and reduce pesticide use", funded by "Regional Competitiveness", 2007-2011; CCI 2007HR161PO001; Instrument of Pre-Accession Assistance; Contract no.: IPA 2007/HR/16IPO/001-040511 (2014-2015). During her PhD, she participated in a scientific and professional pre-doctoral training at Ghent University, Faculty of Bioscience Engineering in Ghent, Belgium, from November 2018 to November 2019, where she participated in the Systemic project, Horizon 2020 (2017-2021). She speaks Croatian (mother tongue) and English (advanced). Her scientific interest is in the application of organic and mineral fertilisers in agriculture as well as foliar fertilisation with the aim of maintaining and increasing the plant nutrient capacity of the soil and increasing the yield and nutritional value of agricultural products. Additionaly, the use of digestate (a by-product of biogas plants) in fertilisation as a partial or complete substitute for mineral fertilisers. Among other things, it has expertise in the areas of soil science and plant protection. She has participated in 10 national and international congresses and published 15 A1 papers as author or co-author (five A1 papers).

Scientific journal articles:

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Supplementary/Appendix

Table 1. ANOVA sliced per treatment for ammonia, nitrate, total mineral nitrogen and nitrogen release

Treatment	Time								
		NH_4^+		NO_3^-		Total min N		N rel	
1	0	4.56	ab	10.64	e	15.20	c	0.00	c
1	20	9.34	a	14.57	d	23.91	ab	1.83	ab
1	40	8.50	a	17.69	c	26.20	bc	2.01	ab
1	60	1.14	b	18.46	c	19.60	c	1.50	b
1	80	3.53	ab	20.33	b	23.86	ab	1.83	ab
1	100	5.68	ab	23.33	a	29.02	a	2.22	a
1	120	4.16	ab	24.24	a	28.40	a	2.18	a
2	0	33.84	a	36.47	f	70.31	ab	0.00	c
2	20	21.01	b	41.04	e	62.05	c	73.82	b
2	40	15.32	c	52.88	d	68.20	ab	81.31	ab
2	60	7.97	d	58.05	c	66.03	bc	89.87	a
2	80	5.05	e	61.87	bc	66.92	bc	83.35	ab
2	100	5.70	e	62.53	ab	68.23	ab	75.89	b
2	120	6.60	de	66.34	ab	72.94	a	86.21	a
3	0	18.08	a	10.64	f	28.72	d	0.00	e
3	20	6.98	b	22.20	e	29.18	d	10.09	d
3	40	6.81	b	27.24	d	34.05	c	15.02	cd
3	60	3.36	d	29.74	cd	33.10	c	25.84	a
3	80	3.23	d	32.16	bc	35.39	bc	22.07	ab
3	100	4.59	cd	33.57	b	38.16	b	17.49	bc
3	120	5.43	bc	37.30	a	42.73	a	27.42	a
4	0	6.12	a	10.64	f	16.76	d	0.00	b
4	20	6.82	a	15.86	e	22.68	c	-2.36	b
4	40	6.80	a	18.45	de	25.25	c	-1.83	b
4	60	1.65	c	19.89	cd	21.54	c	3.75	b
4	80	1.94	c	23.25	bc	25.19	c	2.57	b
4	100	4.83	b	25.88	ab	30.71	b	3.26	b
4	120	6.51	a	28.77	a	35.28	a	13.24	a
5	0	22.12	a	10.64	e	32.76	e	0.00	d
5	20	7.75	bc	31.13	d	38.88	d	28.99	c
5	40	6.56	c	35.12	c	41.68	cd	29.99	c
5	60	7.16	c	38.47	b	45.62	b	50.39	a
5	80	3.86	d	39.44	b	43.30	bc	37.64	b
5	100	4.91	d	40.68	b	45.59	b	32.10	bc
5	120	8.77	b	45.21	a	53.99	a	49.54	a

6	0	21.77	a	21.48	e	43.25	c	0.00	d
6	20	14.03	b	29.24	d	43.27	c	38.16	c
6	40	8.91	c	37.01	c	45.92	bc	38.87	c
6	60	7.31	d	40.32	b	47.63	b	55.24	a
6	80	4.88	e	42.45	b	47.34	b	46.27	b
6	100	4.83	e	42.36	b	47.19	b	35.81	c
6	120	8.59	c	46.48	a	55.07	a	52.56	ab
7	0	28.26	a	22.29	e	50.54	c	0.00	e
7	20	14.34	b	37.17	d	51.51	bc	53.38	cd
7	40	10.48	c	45.07	c	55.55	b	56.77	bcd
7	60	7.80	d	48.31	bc	56.11	b	70.62	a
7	80	4.94	e	50.85	ab	55.79	b	61.74	abc
7	100	4.59	e	50.71	ab	55.30	bc	50.83	d
7	120	7.78	d	54.75	a	62.53	a	66.00	ab

Note. Different letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK). NH_4^+ - ammonia; NO_3^- - nitrate; total min N-total mineral nitrogen; N rel- nitrogen release.

Table 2. ANOVA sliced per time for ammonia, nitrate, total mineral nitrogen and nitrogen release

Treatment	**Time**								
		NH_4^+		**NO_3^-**		**Total min N**		**N rel**	
0	1	0.00		0.00		0.00		0.00	
0	2	0.00		0.00		0.00		0.00	
0	3	0.00		0.00		0.00		0.00	
0	4	0.00		0.00		0.00		0.00	
0	5	0.00		0.00		0.00		0.00	
0	6	0.00		0.00		0.00		0.00	
0	7	0.00		0.00		0.00		0.00	
20	1	9.34	bcd	14.57	e	23.91	d	1.83	f
20	2	21.01	a	41.04	a	62.05	a	73.82	a
20	3	6.98	d	22.20	d	29.18	d	10.09	e
20	4	6.82	d	15.86	e	22.68	d	-2.36	f
20	5	7.75	cd	31.13	c	38.88	c	28.99	d
20	6	14.03	bcd	29.24	c	43.27	c	38.16	c
20	7	14.34	b	37.17	b	51.51	b	53.38	b

40	1	8.50	c	17.69	e	26.20	f	2.01	f
40	2	15.32	a	52.88	a	68.20	a	81.31	a
40	3	6.81	d	27.24	d	34.05	e	15.03	e
40	4	6.80	d	18.45	e	25.25	f	-1.83	f
40	5	6.56	d	35.12	c	41.68	d	29.99	d
40	6	8.91	c	37.01	c	45.92	c	38.87	c
40	7	10.48	b	45.07	b	55.55	b	56.77	b
60	1	1.14	c	18.46	e	19.60	e	1.50	e
60	2	7.97	a	58.05	a	66.03	a	89.87	a
60	3	3.36	b	29.74	d	33.10	d	25.84	d
60	4	1.65	bc	19.89	e	21.54	e	3.74	e
60	5	7.16	a	38.47	c	45.62	c	50.39	c
60	6	7.31	a	40.32	c	47.63	c	55.24	c
60	7	7.80	a	48.31	b	56.11	b	70.62	b
80	1	3.53	c	20.33	e	23.86	e	1.83	f
80	2	5.05	a	61.87	a	66.92	a	83.35	a
80	3	3.23	c	32.16	d	35.39	d	22.07	e
80	4	1.94	d	23.25	e	25.19	e	2.57	f
80	5	3.86	bc	39.44	c	43.30	c	37.64	d
80	6	4.88	ac	42.45	c	47.34	c	46.27	c
80	7	4.94	a	50.85	b	55.79	b	61.74	b
100	1	5.68	a	23.33	e	29.02	e	2.22	e
100	2	5.70	a	62.53	a	68.23	a	75.89	a
100	3	4.59	a	33.57	d	38.16	d	17.49	d
100	4	4.83	a	25.88	e	30.71	e	3.26	e
100	5	4.91	a	40.68	c	45.59	c	32.10	c
100	6	4.83	a	42.36	c	47.19	c	35.81	c
100	7	4.59	a	50.71	b	55.30	b	50.83	b
120	1	4.16	e	24.24	f	28.40	f	2.18	f
120	2	6.60	bcd	66.34	a	72.94	a	86.21	a
120	3	5.43	de	37.30	d	42.73	d	27.42	d
120	4	6.51	cd	28.77	e	35.28	e	13.24	e
120	5	8.77	a	45.21	c	53.99	c	49.54	c
120	6	8.59	ab	46.48	c	55.07	c	52.56	c
120	7	7.78	abc	54.75	b	62.53	b	66.00	b

Note. Different letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK). NH_4^+ - ammonia; NO_3^- - nitrate; total min N-total mineral nitrogen; N rel- nitrogen release

Table 3. Two-way ANOVA fresh and dry biomass yield

Treatment	Treatment			
	Fresh biomass		Fresh biomass	
1	29.61	d	12.71	e
2	39.61	a	17.35	a
3	33.71	bc	14.61	cd
4	32.82	c	14.37	d
5	33.16	c	14.43	d
6	36.46	ab	18.82	bc
7	37.45	a	16.55	ab

Note. Different letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK).

Table 4. Two-way ANOVA results for the effects of year and plant stage on fresh and dry biomass yield

Year	Vegetative stage	Year*vegetative stage			
		Fresh biomass		Fresh biomass	
2018	1	0.70	e	0.09	c
2018	2	54.81	b	20.32	b
2018	3	37.25	d	22.92	a
2019	1	1.01	e	0.12	c
2019	2	66.49	a	23.62	a
2019	3	47.83	c	23.66	a

Note. Different letters indicate significant differences between treatments ($p < 0.001$). 1-vegetative stage V4 or four fully developed leaves; 2- reproductive stage R5 or dent stage (biomass); 3-reproductive stage R6 or phisiological maturity.

Table 5. Two-way ANOVA results for the effects of treatment and plant stage on fresh and dry biomass yield

Treatment	Vegetative stage	Treatment*vegetative stage			
		Fresh biomass		Dry biomass	
1	1*	0.62	h	0.08	g
2	1*	0.79	h	0.10	g
3	1*	0.85	h	0.10	g
4	1*	0.93	h	0.11	g
5	1*	0.91	h	0.11	g
6	1*	0.85	h	0.10	g
7	1*	1.06	h	0.12	g
1	2*	52.92	cd	19.17	f
2	2*	69.84	a	24.95	abc
3	2*	59.00	bc	21.13	def
4	2*	56.14	c	20.34	ef
5	2*	58.00	bc	21.12	def
6	2*	64.11	ab	23.47	bcd
7	2*	64.51	ab	23.62	bcd
1	3*	35.28	g	18.88	f
2	3*	48.21	de	27.01	a
3	3*	41.29	fg	22.61	cde
4	3*	41.38	fg	22.65	cde
5	3*	40.55	fg	22.08	de
6	3*	44.41	ef	23.90	bcd
7	3*	46.79	def	25.89	ab

Note. Different letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizer NPK 15-15-15 + CAN 27%N (NPK+CAN); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-mixture of mineral fertilizer with solid fraction of digestate (SFD+NPK); 7-mixture of mineral fertilizer with liquid fraction of digestate (LFD+NPK). 1*-vegetative stage V4 or four fully developed leaves; 2*- reproductive stage R5 or dent stage (biomass); 3*-reproductive stage R6 or phisiological maturity.