Inland Norway
University of
Applied Sciences

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Hesam Mousavi

Nitrogen-Enriched Organic fertilizer's (NEO) effects on crop yields, soil functions, and species

PhD in Applied Ecology and Biotechnology
2023

Hesam Mousavi

Nitrogen-Enriched Organic fertilizer's (NEO) effects on crop yields, soil functions, and species

Ph.D. Thesis

2023

Applied Ecology and Biotechnology

Inland Norway
University of
Applied Sciences

Printed by: Flisa Trykkeri A/S

Place of publication: Elverum

PhD Thesis in Applied Ecology and Biotechnology no. 35

ISBN printed version: 978-82-8380-452-2

ISBN digital version: 978-82-8380-451-5

ISSN printed version: 2703-819X

ISSN digital version: 2703-8181

Abstract

In an era of population growth, increasing food prices, scarcity of arable land, and environmental degradation of farmlands, demand for novel solutions has emerged. This entails introducing innovative fertilizer products designed to mitigate their environmental footprint. Synergized with complementary strategies, these innovations can bolster food production while safeguarding the food production system's sustainability. Nitrogen Enriched Organic fertilizer (NEO) is produced using a new method, where dinitrogen (N_2) is captured from the air and through a plasma process mixed with bio-based fertilizers as nitrate (NO_3^-) and nitrite (NO_2^-). However, a thorough product assessment is necessary to unveil potential adverse effects before introducing it to the global markets. In this context, our research has centered on examining the fertilizer's impact on soil fauna activity (measured through substrate breakdown), the abundance of key soil species (springtails and earthworms), as well as critical processes like nitrification and nutrient uptake (yield). Different fertilization regimes were employed, including mineral fertilizer, NEO, untreated biobased fertilizers, and no fertilizer across various experimental setups. Regarding soil fauna feeding activity, there were discrepancies between our two studies. However, we observed a tendency for higher feeding activity in unfertilized soil or under lower fertilization amounts, irrespective of fertilizer type. However, the initial perturbative effect of fertilization on soil fauna feeding activity subsided within a few weeks after application. Likewise, NEO and other fertilizers demonstrated no detrimental effects on the abundance and weight of earthworms or the abundance of springtails. The study also investigated the impact of NEO on soil nitrification potential and observed that although NEO initially stimulated nitrification rates in controlled settings, this effect did not persist ≈ six months after fertilization in the field. Concerning crop yields, while yielding slightly less grass than mineral fertilizers under controlled conditions with equivalent N-min input, NEO exhibited a grain yield approximately 20% lower than mineral fertilizer in the field. Albeit, NEO unveiled an advantage, yielding 20–30% more than the original cattle slurry supply. This signifies a noteworthy enhancement in crop productivity, achieved solely through using electricity and cattle slurry as inputs. In brief, the explorations did not detect any harmful effects of NEO on soil functions and key species, while improved crop yields than the feedstock from which it was derived. Thus, our research findings demonstrate that NEO constitutes a meaningful contribution despite its incremental role in transitioning global food production systems toward sustainability.

Keywords: Sustainable agriculture; nitrogen; fertilization; soil health; crop productivity

Sammendrag

I en tid med befolkningsvekst, økende matpriser, knapphet på dyrkbar jord og store miljøutfordringer, kreves det nytenkning. Til dette hører innovative gjødselprodukter med minimal miljøpåvirkning, som sammen med andre tiltak kan bidra til økt matproduksjon samtidig som produksjonssystemets bærekraft ivaretas. Nitrogenberiket organisk gjødsel (NEO) produseres ved hjelp av en ny metode, der nitrogengass (N_2) fanges fra luften og gjennom en plasmaprosess går over til nitrat (NO_3^-) og nitritt (NO_2^-), som deretter kan blandes med biobaserte gjødselstoffer som husdyrgjødsel eller biorest. En grundig testing av NEO er nødvendig for å avdekke eventuelle skadelige effekter på jord, planter og miljø før det lansers på verdensmarkedet. Vårt bidrag i denne sammenheng har vært å undersøke effektene på jordfauna-aktivitet (målt som nedbrytning av et gitt substrat) og på forekomst av nøkkelarter i jord (spretthaler og meitemark), samt på viktige prosesser som nitrifikasjon og næringsopptak (avling). Forskningen omfattet forsøk i felt vekstkammer og laboratorie, og med varierte eksperimentelle oppsett. Forskjellige gjødselregimer ble benyttet, inkludert mineralgjødsel, NEO, ubehandlet organisk gjødsel og ingen gjødsel. Når det gjelder jordfauna-aktivitet var det uoverensstemmelser mellom våre to studier, men så vi en tendens til økt aktivitet i ubehandlet jord eller ved de laveste gjødselmengdene, og dette var uavhengig av gjødseltype. Dette utjevnet seg riktignok kun få uker etter tilførsel. Videre viste NEO og de andre gjødselregimene ingen skadelige effekter, hverken på meitemark eller på spretthaler. Hva gjaldt jordas nitrifikasjonspotensiale så ble det observert økt nitrifikasjon kort tid etter tilførsel av NEO, men dette vedvarte ikke. Etter seks måneder var det ingen forskjell mellom NEO og de andre gjødslingene. Når det gjaldt næringsopptak og plantevekst registrerte vi noe lavere avling etter tilførsel av NEO sammenlignet med mineralgjødsel med tilsvarende næringsinnhold. Samme resultat ble observert i feltforsøkene hvor NEO viste i størrelsesorden 20% lavere avling enn mineralgjødsel med tilsvarende næringsinnhold. Sammenlignet med ubehandlet husdyrgjødsel ga derimot NEO 20-30% høyere avling. Dette viser en betydelig avlingseffekt av plasmabehandlingen. Dette er oppnådd utelukkende ved å bruke elektrisitet og husdyrgjødsel som innsatsfaktorer. Kort sagt fant vi ingen skadelige effekter av NEO på nøkkelarter og viktige funksjoner i jorda, samtidig som avlingene økte sammenlignet med ubehandlet husdyrgjødsel. Våre forskningsresultater viser derfor at NEO representerer et verdifullt bidrag, selv om dette er bare en liten del av hva som må gjøres for å endre de globale matproduksjonssystemer i bærekraftig retning.

Nøkkelord: Bærekraftig landbruk; nitrogen; gjødsling; jordhelse; avlingsproduktivitet.

Contents

List of figures

List of tables

List of papers

Paper 1:

Mousavi, H., Cottis, T., Hoff, G., & Solberg, S. Ø. (2022). Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. Sustainability, 14(4), 2005. doi: 10.3390/su14042005

Paper 2:

Mousavi, H., Cottis, T., Pommeresche, R., Dörsch, P., & Solberg, S. Ø. (2022). Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. Agronomy, 12(10), 2314. doi: 10.3390/agronomy12102314

Paper 3:

Mousavi, H., Solberg, S. Ø., Cottis, T., & Dörsch, P. (2023). Nitrogen-Enriched Organic fertilizer (NEO) elevates nitrification rates shortly after application but has no lasting effect on nitrification in agricultural soils. doi:10.21203/rs.3.rs-2565156/v2.

Paper 4:

Cottis, T., Mousavi, H., & Solberg, S. Ø. (2023). Plasma Treated Cattle Slurry Moderately Increases Cereal Yields. Agronomy, 13(6), 1549. doi: 10.3390/agronomy13061549

1. Background

1.1. Digging up history

The first traces of agriculture go back to 12000 years ago when human beings turned permanent settlers and farmers from nomadic hunter-gatherers (National Geographic Society, 2022). Since plant domestication started, increasing agricultural productivity has fascinated us (Diamond, 2002). Egyptians, Romans, Babylonians, and early Germans are assumed to have used minerals and manure to enhance their farms' productivity. Also, a common farming practice was using wood ash as an amendment (Kiiski, Scherer, Mengel, Kluge, & Severin, 2009). Although presumably Neolithic man used some agricultural amendments, the first modern fertilizer manufactured through chemical means was ordinary superphosphate produced in the early 19th century by exposing bones to sulfuric acid (Russel & Williams, 1977).

The first synthetic nitrogen (N) fertilizer was calcium nitrate, manufactured for the first time in 1903, and from nitric acid generated by the electric arc method (Rouwenhorst, Jardali, Bogaerts, & Lefferts, 2021; Russel & Williams, 1977). Kristian Birkeland, a Norwegian industrialist and scientist, and Sam Eyde, a business partner, were the men behind the process (F. A. Ernst, 1928; Eyde, 1909). However, the Birkeland–Eyde process was energy-inefficient and soon outcompeted by Fritz Haber's method, which was developed in 1913. As a result, the Haber–Bosch method was enhanced and has become dominant in the N fertilizer industry (Rouwenhorst et al., 2021).

The Haber-Bosch method is a process for synthesizing ammonia (NH_3) from hydrogen gas (H_2) and nitrogen gas (N_2) in the presence of iron catalysts at elevated temperatures and pressures. The reaction is exothermic, meaning it releases heat energy, and is described by the following equation: $N_2 + 3H_2 \rightarrow 2NH_3$ (Brightling, 2018; Travis, Travis, & Costa, 2018). The Haber–Bosch method revolutionized the production of ammonia, which is a crucial component in producing fertilizers and many other industrial products (Smil, 2004). This transition allowed the survival of billions of people at the beginning of the 20th century, whereas almost every second person living today owes their existence to this innovation (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008).

1.2. Population growth kicks in

Concurrently with the advancements in the agriculture amendments sector, the world's population more than quadrupled during the last century (Roser, Ritchie, Ortiz-Ospina, & Rodés-Guirao, 2013) (Figure 1). The leading causes for this increment are improved human fertility rates, prolonged life expectancy, and cross-border immigration (Roser M. & Ritchie, 2019; United Nations, 2022). Nonetheless, this is not going to stop. According to the United Nations (UN), the world population is anticipated to increase by almost 2 billion over the next 30 years, from 8 billion to 9.7 billion in 2050 and nearly 10.9 billion in 2100 (United Nations, 2017).

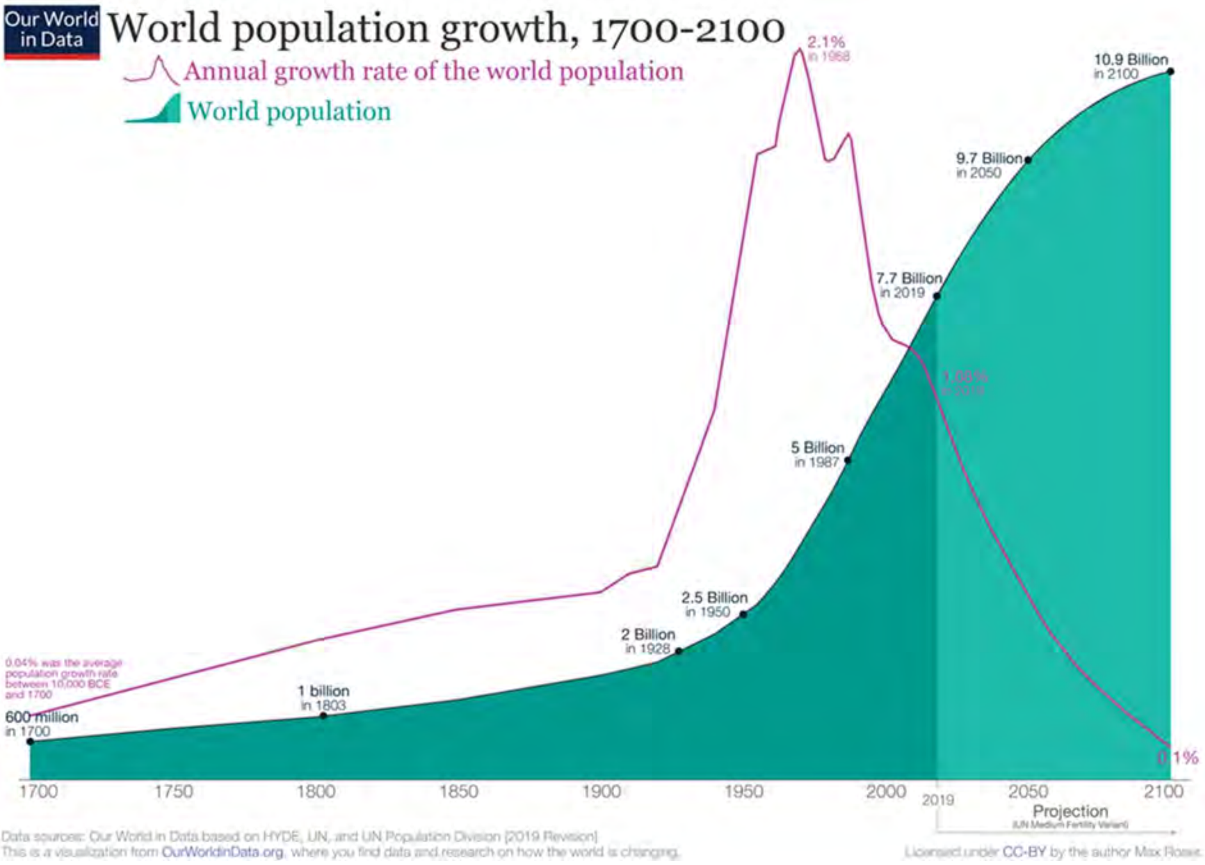


Figure 1: 400 years of world population growth; history and anticipations, Retrieved from (Roser et al., 2013)

Overpopulation has severe environmental consequences (Roser et al., 2013). The two primary impact forms are elevated resource consumption, e.g., land, food, water, air, fossil fuels, and minerals, and increased waste production, e.g., air and water pollutants, toxic materials, and greenhouse gases (Hedberg, 2020).

1.3. Overpopulation and agricultural ecosystems

On the agriculture side, the world's overpopulation occurs in parallel with 12 million hectares of arable land being lost from production annually (Döös, 2002; Prăvălie et al., 2021; United Nations, 2019). Multiple threats exert pressure on the world's soils owing to population growth, economic development, and climate change (Montanarella et al., 2016). Land degradation strongly correlates with food security, whereas 99% of our food is produced in terrestrial systems and only 1% in aquatic systems (Pimentel, 2006). Thus, global arable lands progressively encounter challenges ensuring global food security and the substantial demand for increased food production. The grounds mentioned above imply that providing space, food, and resources sustainably for the future global population is undoubtedly one of our generation's foremost challenges (Roser et al., 2013). Simultaneously, agroecosystems encounter significant societal demands to promote sustainable food production within this context (Augustin et al., 2016; Janker, Mann, & Rist, 2019; Vermeir & Verbeke, 2006). Hence, developing new environmentally friendly fertilizers or carefully utilizing existing fertilizers emerges as potential pathways to attain greater sustainability, equity, and resilience in agroecosystems. These approaches promise to advance global food security and foster a more secure future.

1.4. The elemental power of Nitrogen (N)

Nitrogen (N) is an essential element in agroecosystems. N contributes to several essential processes in plants, e.g., growth, leaf area development, and biomass production. Numerous N-structured molecules, including amino acids, chlorophyll, nucleic acids, ATP, and phytohormones, are engaged in the plants' physiological processes, e.g., carbon and N metabolism, photosynthesis, and protein assimilation (Crawford & Forde, 2002; Frink, Waggoner, & Ausubel, 1999).

The favorable influence of N fertilization on plant productivity is widely explored and well-recognized (Basso et al., 2016; Dong & Lin, 2020; Houlton et al., 2019). N enhances root extension and nutrient uptake (Diaz et al., 2006; Good, Shrawat, & Muench, 2004; Stitt & Krapp, 1999). N deficiency can diminish plants' growth and development (Ding et al., 2005). Thus, N availability is one of the most crucial requirements in plant production (Bondada & Oosterhuis, 2001; Rütting, Aronsson, & Delin, 2018).

N in the biological systems is found in mineral and organic forms (Mann, 1983). Mineral N refers to inorganic forms of N found in soil and fertilizers. Nitrate (NO_3^-) and ammonium (NH_4^+) are the primary mineral N forms, which are readily available to plants because they can be directly up taken through their roots (R. L. Mulvaney, 1996). Mineral N can come from various sources, including mineral fertilizers, organic fertilizers, decomposition of organic matter, and atmospheric deposition (such as N gas converted into nitrate by lightning or industrial processes) (Ghaly & vasudevan ramakrishnan, 2015).

Nitrate (NO_3^-) is a highly mobile form of N in soil. It dissolves in water and can be easily transported to plant roots. Plants can uptake nitrate ions through their root systems, making it a readily available N source for their growth and development (Miller & Cramer, 2005). Ammonium (NH_4^+) is another form of mineral N. It is positively charged and less mobile in soil compared to nitrate (Muratore, Espen, & Prinsi, 2021). Plants can also directly absorb ammonium ions through their roots (Miller & Cramer, 2005). Ammonium is often used as a N source in mineral fertilizers (Marschner, 2002a, 2002b, 2002c).

Mineral N is generally more readily available to plants than organic N (Muratore et al., 2021). Plant roots can directly take up nitrate and ammonium without further microbial decomposition. However, plant mineral N availability depends on soil pH, temperature, and moisture content (Masclaux-Daubresse et al., 2010).

Organic N refers to N in complex organic compounds, such as proteins, amino acids, and organic matter like plant residues, animal manure, and compost. Organic N is not immediately available for plant uptake; it must undergo microbial decomposition to convert it into mineral forms (nitrate or ammonium) that plants can use. Soil bacteria and other microorganisms carry out this decomposition (Eulene Francisco da et al., 2019).

The availability of organic N to plants depends on soil microorganisms' decomposition rate and mineralization of organic matter. Factors such as temperature, moisture, and carbon-to-nitrogen ratio (C/N ratio) in the organic material influence how quickly organic N is converted into mineral forms (Eulene Francisco da et al., 2019).

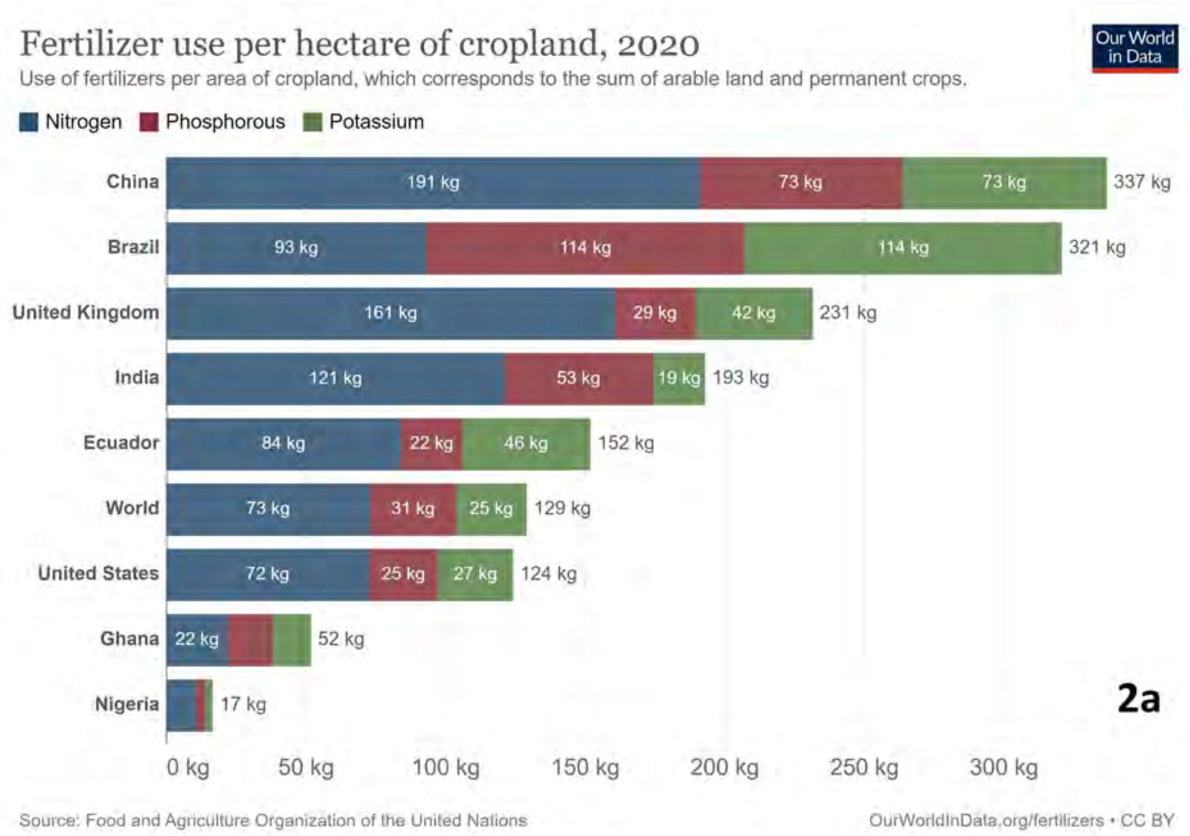
The N content in manure can vary widely depending on the source and management of the manure. For instance, chicken manure typically contains more N than cow or horse manure. On average, poultry manure may contain 3-4% N by weight, while other types might have lower N content, usually ranging from 1% to 2% (Sundermeier, 2016). The N in manure is primarily organic and requires time to mineralize and become available to plants (Masclaux-

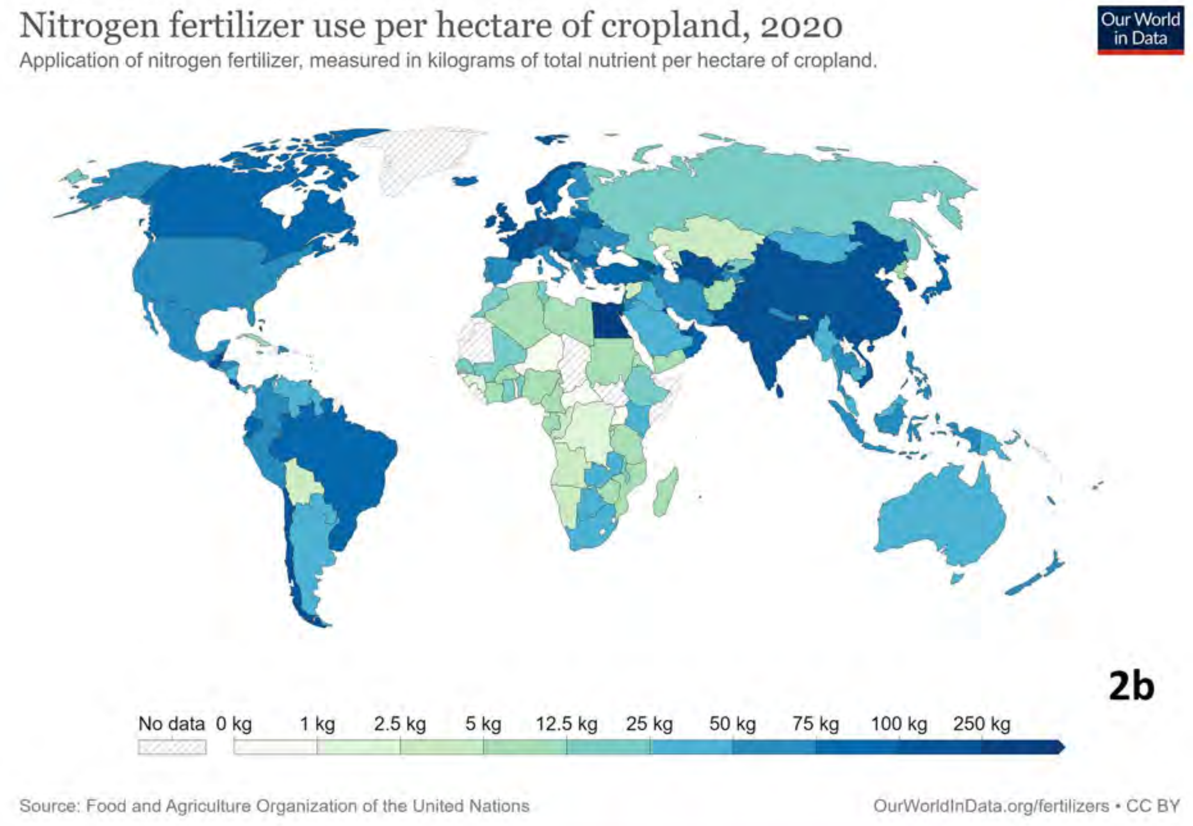
Daubresse et al., 2010). However, mineral fertilizers are fabricated to provide plants with readily available N. Common mineral fertilizers include urea, ammonium nitrate, and ammonium sulfate. These fertilizers can have N content ranging from 10% to 82% (Isleib, 2017). For example, urea typically contains about 46% N.

1.5. The good, the bad, and the ugly

N fertilizers are vital for global food production and food safety. Not so many innovations have changed the history of humankind as much as mineral N. N fertilizers also maintain enormous environmental benefits where a reduced area is used to harvest increased crop yields (Ritchie, 2021). Therefore, N fertilizers contribute to safeguarding forests and preserving natural habitats. However, despite positive effects, overusing N fertilizers could be associated with severe drawbacks (Upendra, Rajan, & Gautam, 2019). The use of N (N) in the agroecosystem has undergone a remarkable transformation over the past few decades. The global input of N has increased exponentially by eight-fold since the 1960s (FAO, 2019; Matson, Parton, Power, & Swift, 1997). This staggering increase in N usage, which is well-illustrated in Figure 2a, is a testament to the technological advancements in agriculture and reflects the growing demand for food production worldwide.

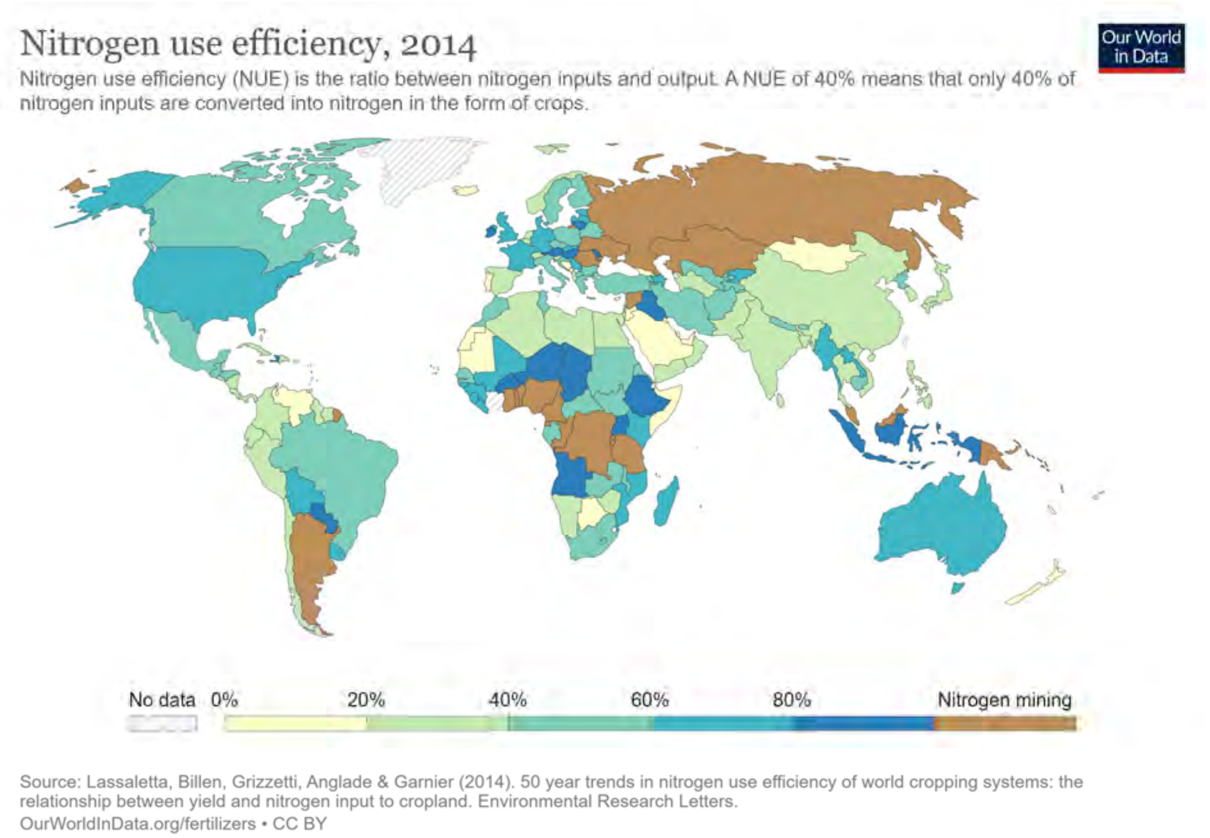
Interestingly, the geographical distribution of major fertilizer use hubs has significantly shifted over time. In the 1960s, the United States and Western Europe were the dominant players in the fertilizer market. However, this trend has changed dramatically in recent years. As of the early 21st century, Eastern Asia has emerged as the primary hub for fertilizer use (Lu & Tian, 2017) (Figures 2a and 2b).





Figures 2a and 2b: The major global consumers of N fertilizers per hectare cropland 2020, retrieved from FAO stat and OurworldInData.org/fertilizers (FAO, 2022a; Ritchie, Roser, & Rosado, 2022)

Unfortunately, N Use Efficiency (NUE) is a cause for concern worldwide, as depicted in Figure 3. This is compounded by the rising costs of fertilization, coupled with the fact that more than half of the supplemented N is lost to the environment in various forms (Chen, Chen, Tseng, & Tsay, 2020; Lassaletta, Billen, Grizzetti, Anglade, & Garnier, 2014; McAllister, Beatty, & Good, 2012; R. Mulvaney, Khan, & Ellsworth, 2009).



Nitrogen use efficiency, 2014

Nitrogen use efficiency (NUE) is the ratio between nitrogen inputs and output. A NUE of 40% means that only 40% of nitrogen inputs are converted into nitrogen in the form of crops.

Source: Lassaletta, Billen, Grizzetti, Anglade & Garnier (2014). 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. Environmental Research Letters.
OurWorldInData.org/fertilizers • CC BY

Figure 3: Nitrogen Use Efficiency (NUE) worldwide, retrieved from OurworldInData.org/fertilizers.

As a result of the widespread use of mineral fertilizers, there is now a staggering two-fold increase in the amount of mineral N present in biological systems compared to a century ago (United Nations, 2020b). The consequences of such growth are far-reaching, leading to environmental pollution, disruption of natural processes, and significant negative impacts on biodiversity and the climate (European Commission, 2022).

1.6. N fertilization and soil life

Although we consume N fertilizers to boost agroecosystems' fertility and profitability, how they influence soil organisms is typically disregarded. Disagreements are yet noticeable even if several studies explored the effect of nutrient manipulation on soil-dwelling organisms (Bünemann, Schwenke, & Van Zwieten, 2006; Siebert et al., 2019). N fertilization imposes variations on soil communities by altering the soil nutrient spectrum and favoring the ecosystem for particular groups (Bell, Asao, Calderon, Wolk, & Wallenstein, 2015). However, soil functional groups' response to frequent mineral N differs notably within environmental and management factors (Geisseler & Scow, 2014).

Mineral fertilizers could enhance soil biodiversity and organic activity by promoting plant productivity, crop residue return, and soil organic matter (Siebert et al., 2019). Similarly, organic amendments, e.g., manure, compost, biosolids, and humic substances, provide C and N resources for soil organisms (Wei et al., 2017). However, in the long run, a surplus of mineral N might acidify the soil and damage soil organisms (Bünemann et al., 2006). Albeit the vulnerability of soil invertebrates to mineral N varies from those of microbial communities (Bünemann et al., 2006).

The susceptibility of soil fauna and invertebrates to elevated N levels varies significantly. For instance, studies indicated a detrimental effect of N on soil organisms' feeding activity in short-term (Siebert et al., 2019) and long term (Pelosi, Boros, van Oort, & Schmidt, 2020; Tao, Slade, Willis, Caliman, & Snaddon, 2016) but positively influenced soil fauna's composition and diversity (Graenitz & Bauer, 2000; Silva et al., 2016; Wahyuningsih, Marchand, Pujianto, & Caliman, 2019). In a separate investigation, the application of cattle slurry led to a temporary decrease in the abundance of springtails, which later partially recovered during the same growing season but did not return to the initial levels (Pommeresche, Løes, & Torp, 2017).

Conversely, some reports highlight the positive impact of fertilizers on soil faunal structure, diversity, and feeding activity (Graenitz & Bauer, 2000; Wahyuningsih et al., 2019), particularly in the case of springtails within the topsoil layer (Silva et al., 2016).

The impact of fertilizers on earthworm populations reveals that a combination of mineral and organic fertilizers exerts a more significant influence than using mineral fertilizer alone, as indicated by multiple studies (C. A. Edwards, 1977; C. A. Edwards & Lofty, 1982; Hendrix, Mueller, Bruce, Langdale, & Parmelee, 1992; Tiwari, 1993) Additionally, one study highlights the positive effect of mineral fertilizer on the abundance of springtails and mites, although it comes at the cost of reduced species diversity (Guðleifsson, 2002).

On the contrary, N-based fertilizers, particularly those rich in ammonium N, have the potential to negatively affect soil biological activity due to soil acidification and alterations in soil functional communities, as documented by various sources (Bünemann et al., 2006; Clive

A Edwards & Bohlen, 1996; Mc Laughlin & Mineau, 1995). Furthermore, the repeated application of mineral fertilizers may suppress specific soil enzymes involved in nutrient cycles, such as the amidase in the N cycle, as observed in prior research (Mc Laughlin & Mineau, 1995). A study indicated a diminishing effect of N input on microbial biomass in grassland soil, i.e., presumably due to restriction in plant species richness, but not in annual cropland soil (Geisseler, Lazicki, & Scow, 2016; Herren et al., 2020; Siebert et al., 2019).

These contrasting findings underscore the significance of exploring the effects of fertilizers on soil biota, especially in the context of innovative fertilizers.

1.7. The fundamental role of soil life

Soil is one of the most critical 'global reservoirs' of biodiversity, home to more than 25 percent of the world's biological diversity (United Nations, 2020a). However, the contribution of life in soil remains vastly underrated. That being said, we estimate that we know nearly 1% of the microorganisms living in the soil (FAO, 2022b). Furthermore, over 40 percent of living organisms in terrestrial ecosystems are linked with soils during their life cycle (United Nations, 2020a) (Figure 4).

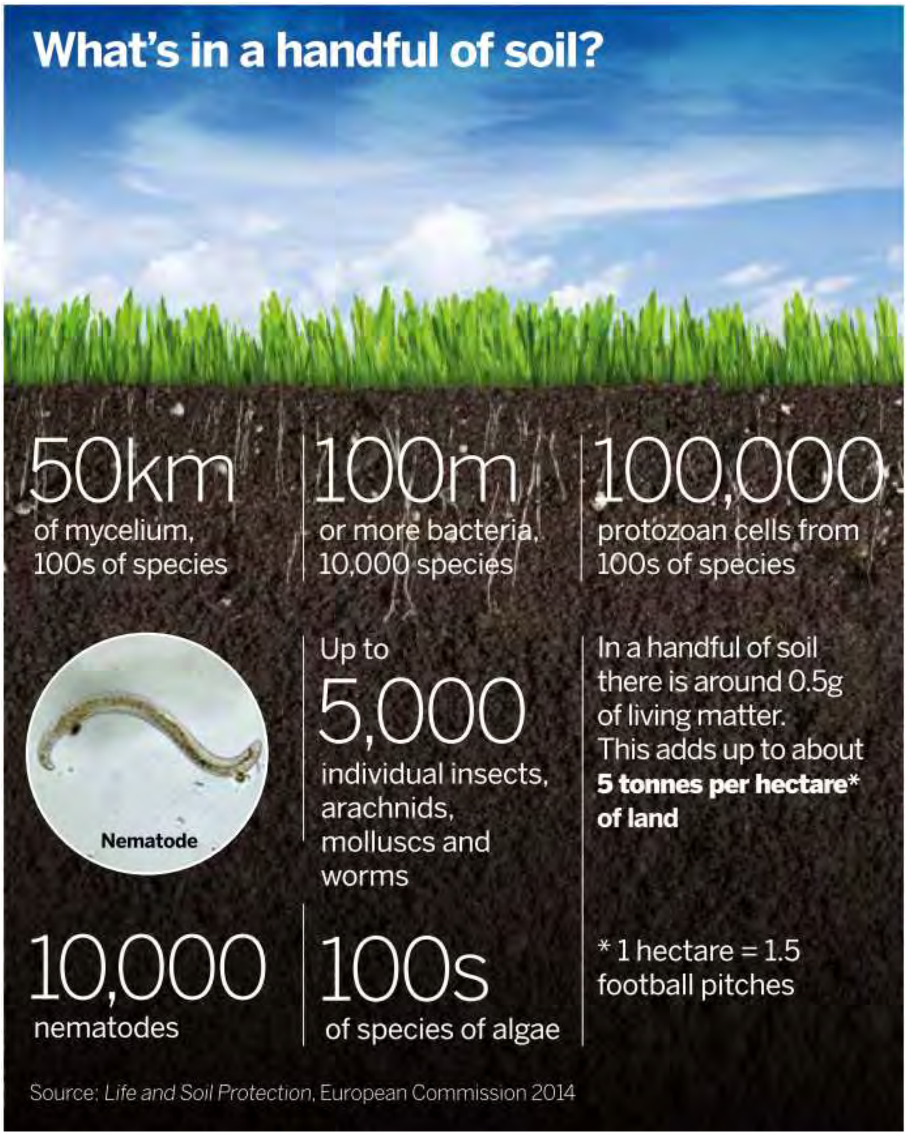


Figure 4: What is living in a handful of soil, retrieved from (European Commission et al., 2014)

The activities of soil organisms are critical for the maintenance of healthy soils. These organisms can alter the soil's physical structure by digging burrows, adding nutrients to the soil by decomposing dead leaves, and helping regulate the populations of other soil organisms (Bottinelli, Hedde, Jouquet, & Capowiez, 2020). Soil organisms are also essential for most soil ecosystem services, including providing healthy food and improving agricultural output, water filtration, carbon sequestration, the degradation of target contaminants, and the medicine supply.

Soil fauna, a vital group of primary heterotrophs in soil ecosystems, promotes bacterial and fungal diversity and activity within soils (Hättenschwiler, Tiunov, & Scheu, 2005). Invertebrates profoundly impact nutrient cycling by directly consuming plant materials and organic substrates. These materials' physical breakdown or comminution accelerates their decomposition process by increasing the surface area of plant structural materials and exposing the inner cytoplasm, making it more accessible to microbial activity (Coleman & Wall, 2015).

Furthermore, invertebrates' feeding habits often lead to N translocation from the soil to the substrate in the form of fecal material and through fungal hyphae. Grazing by invertebrates is vital in disseminating microbes from one organic source to another. Many of these microbes adhere to the exoskeletons and cuticles of invertebrates and can survive their journey through the digestive tracts. This mechanism further contributes to the interconnectedness of soil ecosystems, facilitating the movement and distribution of crucial microorganisms (Coleman & Wall, 2015; Meier, Scherrer, & Honegger, 2002).

In agroecosystems, soil organisms transform nutrients, making nutrients accessible to plants (Coleman & Wall, 2015). In addition, certain soil microbiota can reduce agricultural costs and reliance on mineral N and P fertilizers and improve soil fertility, agricultural output, and environmental sustainability (FAO, 2022b).

Due to numerous links between above and belowground, soil biodiversity controls the primary processes allowing life to exist on earth. Nonetheless, the loss of soil biodiversity is deemed one of the most serious global threats to soils in many regions of the globe (FAO, 2022b). Therefore, it is vital to incorporate policies that reduce soil degradation and safeguard soil biodiversity from threats into biodiversity conservation and ecosystem restoration efforts at all levels.

1.8. Sowing the seeds of change: Practices, policies, and projections

Conserving soil biodiversity requires advanced monitoring systems, standardized procedures for sampling and analysis, and global cooperation to compile datasets for scientific evidence. In addition, research into soil communities can help us understand the complex relationships between soil organisms and their environment and develop effective conservation strategies. Investing in these efforts will promote sustainable land use practices and ensure the well-being of the planet and all its inhabitants.

Farmers and land users/owners should employ sustainable soil management practices to prevent and reduce soil biodiversity loss. Unfortunately, N fertilizers are over and under-applied in many countries worldwide, with a 100-fold distinction between countries (Mueller et al., 2012; Ritchie, 2021). For instance, a large-scale study in China indicated that modest interventions could produce massive improvements; for ten years, 21 million smallholder farmers followed recommended management practices, including reduced N fertilization by 15%, and remarkably, these adjustments were coupled with a notable increase in average yields by 11%. Besides the economic profit of US$ 12.2 billion, the environmental impact of farming has decreased just by educating and training farmers on proper management practices (Cui et al., 2018).

While educating farmers on sustainable management practices is essential, developing and implementing innovative strategies and guidelines is equally important to help steer the agricultural sector toward a more sustainable future. One example is the United Nations Environment Program's "Halve Nitrogen Waste" campaign, which aims to reduce N waste by 50% by 2030. This campaign can generate a staggering $100 billion economic return globally (50% of the global mineral fertilizer market). The European Commission's "Farm to Fork strategy" is another example of a comprehensive plan to promote sustainability in the agricultural sector. This strategy takes a holistic approach, encompassing everything from reducing pesticide use to promoting organic farming practices (European Commission, 2022; United Nations, 2020b). Therefore, by setting clear goals and targets, such a strategy provides a roadmap for the entire agricultural sector to work toward a more sustainable future as part of the European Green Deal for achieving climate neutrality by 2050.

One more practical approach is developing sustainable, organic-based agricultural amendments. Recently, affordable, renewable energy growth has drawn interest in N fixation by the neglected Birkeland–Eyde plasma technology (Winter & Chen, 2021). By utilizing renewable energy sources, non-thermal processes, and reducing carbon footprint and waste, plasma-backed N fixation maintains milder operation conditions and a greener environmental profile than the Haber-Bosch process. It also enables decentralized fertilizer production (Li, Medrano, Hessel, & Gallucci, 2018). However, despite its potential, this technology still holds shortcomings. These include scale-up challenges, high initial investment costs, lower ammonia yield compared to traditional methods, safety concerns, and negative environmental impacts, specifically when fossil fuel-based electricity is utilized (Tsonev, O'Modhrain, Bogaerts, & Gorbanev, 2023).

A study indicated that benefiting from the recent developments, the energy consumed for generating NO_X using plasma technology could be comparable with the Haber-Bosch process. However, further plasma reactor improvements and efficient plasma–catalyst coupling are needed (Rouwenhorst et al., 2021). After all, using plasma technology, the Norwegian company N2 Applied (Asker, Norway) has been inspired to develop a unit for enriching organic amendments like manure and biogas digestate with atmospheric N (N2 Applied, 2022).

1.9. The rise of Nitrogen-Enriched Organic fertilizer (NEO)

Nitrogen oxide assimilation through plasma processes has been a well-established concept for over a century. However, incorporating nitrate enrichment in organic fertilizers is a recent development (Ingels & Graves, 2015; N2 Applied, 2022). However, one of the main challenges in this field is the high energy consumption of plasma reactors. Despite this, the production of reactive N has significantly improved compared to 100 years ago.

The recent technological advancements enabled N2 Applied to develop a patented unit that uses a plasma-arc process to enrich atmospheric N into the organic slurry or biogas digestate. The warm or cold plasma process fixes N from the air as nitrogen oxides (NO_x), which in reaction with water results in the formation of nitrous (HNO_2) and nitric (HNO_3) acids, combined with slurry or biogas digestate and lowering the pH and microbial activity of the mixture (Graves, Bakken, Jensen, & Ingels, 2019). The final product is NEO, a biobased fertilizer rich in nitrogen (with approximately double the N content compared to the native feedstock) that has been acidified.

N2 Applied claims that NEO's high acidity and lower biological activity minimize greenhouse gas emissions compared to conventional, organic-based fertilizers. Moreover, NEO-treated slurries are filtered and highly liquefied, making them easier to manage in precision agriculture (N2 Applied, 2022). N2 Applied also asserts that their unit provides a novel solution to the conventional fertilizer production model by decentralizing the production process. As a result, farmers can invest in N2 units and produce fertilizer on their farms.

Besides the company's ambition to enhance the agricultural industry with advanced technology, its goal is to reduce reliance on traditional fossil-fuel-based N fertilizers. Nonetheless, N2 Applied's success relies strongly on the availability of low-cost energy sources and the performance of the fertilizer product in the field, which need to be further elucidated. As such, guaranteeing the fertilizer meets the required quality standards is paramount. It must deliver the expected crop yields while remaining harmless to the soil system. Only then can N2 Applied claim that their technology revolutionizes farming practices and promotes sustainable agriculture.

2. Scientific Aims and Methods

2.1. Grounds

Since NEO represents an innovative and ambitious product, it is imperative to conduct precautionary assessments. These assessments serve to uncover its benefits and potential drawbacks, as well as to verify its environmental and agricultural safety. Additionally, for a holistic understanding, it is crucial to evaluate the fertilizer's impact on plant yields thoroughly and to make comparisons against the conventional alternatives used by farmers.

From a soil health standpoint, the elevated nitrite content and acidity of NEO raise concerns, as they could potentially lead to the formation of toxic compounds like radicals and other oxidative byproducts. Although there is currently no evidence pointing to potential disruptions in these aspects, it remains crucial to investigate and elucidate potential drawbacks before the product's global market availability.

Accordingly, as part of the fine-tuning process in collaboration with N2 Applied, we aim to assess NEO's compatibility with soil-living organisms and ensure it delivers satisfactory plant yields. To achieve this, we have undertaken extensive studies to map and compare the effects of NEO with those of other conventional fertilizers (**Paper 1, paper 2, paper 3, paper 4**) (Cottis, Mousavi, & Solberg, 2023; Mousavi, Cottis, Hoff, & Solberg, 2022; Mousavi, Cottis, Pommeresche, Dörsch, & Solberg, 2022; Mousavi, Solberg, Cottis, & Dörsch, 2023). By proactively identifying potential downsides, the company can implement necessary adjustments to NEO, thereby ensuring its safety and effectiveness.

For our studies, we hypothesized that utilizing NEO as an amendment in agriculture would not cause any detrimental effects on soil functions and species compared to other conventional fertilizers commonly used in modern-day agriculture. Furthermore, our hypothesis posited that employing an equivalent quantity of N-min in NEO could produce comparable crop yields to mineral fertilizer.

2.2. Approaches

Multiple studies were conducted across diverse settings, encompassing field conditions, growth chambers, and laboratories. These comprehensive investigations aimed to thoroughly examine the potential impact of NEO on various soil-dwelling organisms and crop yields, facilitating a deeper understanding of the ecological consequences of this phenomenon. The core focus of the soil-related investigations was to evaluate the impact of NEO on a wide range of soil-inhabiting communities, including mesofauna, macrofauna, and microbes. We utilized the following methodologies in our investigations to accomplish our goals:

i. Evaluating changes in soil nutrient cycling, e.g., the biological activity of soil fauna through feeding activity (**Paper 1 and Paper 2**).

ii. Evaluating changes in abundance and biomass of key species in the soil, e.g., mesofauna springtails (*Collembola sp.*) and macrofauna, earthworms (*Lumbricus sp.*) (**Paper 2**).

iii. Evaluating changes in the soil N cycle, e.g., nitrification performed by soil nitrifying microbes (**Paper 3**).

iv. Evaluating the NEO's impact on crop yields (**Paper 1 and Paper 4**).

2.3. Methods

2.3.1. Evaluating changes in soil nutrient cycling through soil fauna feeding activity (Paper 1 and Paper 2)

One of the functional activities of the soil fauna, namely feeding activity, is fundamental for decomposition and nutrient cycling in soil (Hamel, Schellenberg, Hanson, & Wang, 2007; Jänsch, Scheffczyk, & Römbke, 2017; Kratz, 1998; Schinner, Öhlinger, Kandeler, & Margesin, 1996). As a practical, rapid, and duplicable method, the Bait-lamina strip method (Terra Protecta GmbH, Berlin, Germany) (Terra Protecta, 2020) was used for evaluating soil fauna feeding activity in our studies (Hamel et al., 2007; Törne, 1990) (Figure 5).

Figure 5: Bait-lamina strips used to investigate the soil fauna feeding activity.

The method has been used in screening different soil management practices and provides valuable insights into the feeding activity of soil fauna (Hamel et al., 2007). The method involves perforated PVC strips (1 mm × 6 mm × 120 mm) with 16 1.5 mm diameter holes located 5 mm apart. These holes are filled with bait substrates containing 5% activated carbon, 25% wheat fiber, and 70% cellulose powder (ISO18311(E), 2015). After exposure to the soil for a certain period, the degree to which substrate is consumed in the holes indicates the feeding activity of soil fauna, with soil microbes having a negligible effect (Gardi et al., 2009; Rożen, Sobczyk, Liszka, & Weiner, 2010; Simpson, Slade, Riutta, & Taylor, 2012; Terra Protecta, 2020).

For this study, the pot experiments were conducted in a growth chamber at Inland Norway University of Applied Sciences using perforated pots (13 x 13 x 18 cm) filled with 2.5 L of field soil (Figure 6). Four replicates were used to randomize each fertilizing treatment. Two trials were performed **(Paper 1)**, with trial one consisting of 14 fertilizing treatments and trial two with 11 treatments to investigate and compare the impact of different fertilizer treatments on soil fauna feeding activity under controlled conditions.

The treatments included five fertilizing regimes: no fertilizer, different amounts of mineral fertilizer (Yara, 2021b), three types of NEO (N2 Applied), organic fertilizer (untreated cattle slurry), and organic fertilizer with different amounts of N in mineral fertilizer (Yara, 2021a). The two types of mineral fertilizers used were chosen to create a similar N-min profile to NEO (nitrate and ammonium components) in the mineral fertilizer and organic + mineral fertilizer treatments.

Figure 6: Experimental setup in the growing chamber investigating the effects of different fertilization regimes on soil fauna feeding activity.

In trial one, the range of fertilization was from no fertilizer to a maximum of 235 kg N-min ha^{-1}. In contrast, in trial two, it was from no fertilizer to a maximum of 175 kg N-min ha^{-1}. In addition, the Yara mineral fertilizers were added as pellets, while the untreated slurry and all NEO types were added in liquid form.

The fertilizing treatments in trial one were (1) No fertilizer, (2) Mineral fertilizer 115 kg N-min ha^{-1}, (3) Mineral fertilizer 145 kg N-min ha^{-1}, (4) Mineral fertilizer 175 kg N-min ha^{-1}, (5) Mineral fertilizer 205 kg N-min ha^{-1}, (6) Mineral fertilizer 235 kg N-min ha^{-1}, (7) NEO type A 175.4 kg N-min ha^{-1}, (8) NEO type B 175.4 kg N-min ha^{-1}, (9) NEO type C 175.4 kg N-min ha^{-1}, (10) Organic fertilizer 73 kg N-min ha^{-1}, (11) Organic fertilizer + MF 115 kg N-min ha^{-1}, (12) Organic fertilizer + MF 145 kg N-min ha^{-1}, (13) Organic fertilizer + MF 175 kg N-min ha^{-1}, and (14) Organic fertilizer + MF 205 kg N-min ha^{-1}.

The fertilizing treatments in trial two were (1) No fertilizer, (2) Mineral fertilizer 60 kg N-min ha^{-1}, (3) Mineral fertilizer 80 kg N-min ha^{-1}, (4) Mineral fertilizer 115 kg N-min ha^{-1}, (5) Mineral fertilizer 135 kg N-min ha^{-1}, (6) Mineral fertilizer 155 kg N-min ha^{-1}, (7) Mineral fertilizer 175 kg N-min ha^{-1} (8) NEO type A 175.4 kg N-min ha^{-1}, (9) NEO type B 175.4 kg N-min ha^{-1}, (10) NEO type C 175.4 kg N-min ha^{-1}, and (11) Organic fertilizer 73 kg N-min ha^{-1}.

Studies used three types of NEO from different production batches and methods. They differed in acidity, nitrate, and nitrite content. NEO A had a pH of 5.42 and contained 1530 mg/L NH_4^+, 800 mg/L NO_2^-, and 1180 mg/L NO_3^-, while NEO B had a pH of 5.35 and contained 1480 mg/L NH_4^+, 777 mg/L NO_2^-, and 1250 mg/L NO_3^-. NEO C had a pH of 4.24 and contained 1100 mg/L NH_4^+, 444 mg/L NO_2^-, and 1910 mg/L NO_3^-. The cattle slurry used had a pH of 7.13 and contained 1320 mg/L of NH_4^+. The untreated manure target amount was 55 tons ha$^{-1\,(}$1.33 kg plant-available N). However, after processing by the N2 Applied unit, the final product (NEO) had a plant-available N content of 3.51 kg per ton.

Furthermore, another trial (**paper 2**) was performed, differing from the first two trials regarding the amount of mineral and organic fertilizers and the type of NEO (Type D) used. This trial consisted of five fertilization regimes distributed in seven fertilization treatments: no fertilizer; mineral fertilizer (Yara Mila 18-3-15); NEO type D (N2 Applied); organic fertilizer (untreated cattle slurry); and organic fertilizer + mineral fertilizer (Yara Liva 16-0-0). NEO and untreated slurry were applied in liquid form, while Yara Mila and Yara Liva were pelleted. The two types of mineral fertilizers used were chosen to create a similar N-min profile to NEO (nitrate and ammonium components) in the mineral fertilizer and organic + mineral fertilizer treatments.

Treatments were (1) no fertilizer, (2) mineral fertilizer 73 kg N-min ha^{-1}, (3) mineral fertilizer 175 kg N-min ha^{-1}, (4) NEO type D 73 kg N-min ha^{-1}, (5) NEO type D 175 kg N-min ha^{-1}, (6) organic fertilizer 73 kg N-min ha^{-1}, (7) organic fertilizer + MF 175 kg N-min ha^{-1}.

NEO type D had a pH of 5.22 and contained 1746 mg L^{-1} NH_4^+, 1131 mg L^{-1} NO_2^-, and 1562 mg L^{-1} NO_3^-, totaling 4439 mg L^{-1} N. The untreated slurry had a pH of 7.32, containing 1804 mg/L NH_4^+ and 149 mg L^{-1} NO_2^-, totaling 1953 mg L^{-1} N. Therefore, we targeted a slurry amount of 55 tons ha^{-1}. A ton of untreated slurry contained 1.95 kg of plant-available N, while each ton of NEO contained 4.44 kg of plant-available N.

The soil used in these studies was sandy clay loam with over 10% clay and 4.5% soil organic matter, as analyzed by Eurofins before the experiment. The soil pH was high (pH = 7.4), while the phosphorus status was normal (P-AL = 11 mg/100 g), and the potassium status was low (K-AL = 5 mg/100 g). The soil had a field water capacity of 33.6% of soil volume and a pore capacity of 41.4%.

We conducted preliminary tests to determine appropriate intervals for bait-lamina sampling in a pot experiment. Our experiments showed that the proportion of bait consumed in the strips ranged from 3% to 29% after four weeks of soil fauna feeding activity. Eight weeks into the experiment, we noticed some strips that had 100% of their holes empty, suggesting 100% feeding activity. So, we decided that seven weeks would be a suitable evaluation duration.

In our experiments, we planted three Bait-lamina strips diametrically in each pot (replicate) after sowing (week 1) to assess and compare the early effect of fertilization on soil fauna feeding activity (**Paper 2**). To assess the mid-term impact, we removed the first set of strips after seven weeks and replaced them with the second group in the same order (**Paper 1 and Paper 2**). To assess the late effects of fertilizing soil fauna feeding activity, we removed the second set and inserted the third set at week 14 and week 21 after sowing/fertilizing (**Paper 1 and Paper 2, respectively**).

Thirty-six Italian ryegrass seeds (*Lolium multiflorum Lam.*), variety 'Barpluto' (NAK Nederland/Ref. DE148-214011) were sown in the pots, and plant growth and irrigation were maintained until the experiment's termination to replicate field circumstances. The plants were harvested twice: once six weeks after sowing/fertilization and again 14 weeks after sowing/fertilization. The yield results represent the total from these two harvesting occasions.

We visually checked the strips during each sampling for the disappearance of the bait substrate and scored each strip according to its degree of disappearance: empty (score 1), partly empty (score 0.5), or filled (score 0) (Siebert et al., 2019). With the highest percentage of feeding activity (all 16 empty holes, 100 %), each empty hole (score 1) was equal to 6.25% feeding activity.

2.3.2. Evaluating changes in abundance of soil mesofauna, e.g., springtails (*Collembola sp.*) (Paper 2)

Springtails are soil-dwelling creatures living in different soil layers depending on moisture (Verhoef & Brussaard, 1990). They have diverse forms and diets, ranging from grazing on fungi, algae, and bacteria to consuming plant detritus or organic substances (Hopkin, 1997). Springtails are critical bio-indicators of soil health, particularly in shallow soils, and play a significant role in the food chain as prey for other arthropods (Schinner et al., 1996).

To study the abundance of springtails, we conducted sampling in two field trials that received different fertilization treatments, including NEO, mineral fertilizer, organic fertilizer, and no fertilizer, one year before the first sampling. The trials were conducted on a cereal field at the Inland Norway University of Applied Sciences' Blæstad experimental farm (60°49′11.7″ N 11°10′48.4″ E) (Figure 7) and on a grass field in Stjørdal, Trøndelag (63°20′33.4″ N 10°17′56.9″ E). The experimental design was a conventional randomized complete block design with four replicates for each trial.

Figure 7: The experimental cereal field used to conduct experiments investigating the effects of different fertilization regimes on the abundance of soil mesofauna (springtails) and soil nitrification potential.

The fertilization regimes for both fields were identical, and the fields were fertilized for two consecutive years. The fertilizers used were mineral fertilizer (Yara Mila 18-3-15) (Yara, 2021b); NEO type B produced from cattle slurry at the Norwegian University of Life Sciences (NMBU) research barn (N2 Applied, 2022); organic fertilizer (untreated cattle slurry, same as the slurry used for the NEO production); and no fertilizer. The grass field underwent bi-annual fertilization - first in the early spring to stimulate growth and again after the initial harvest to replenish vital nutrients - while the cereal field was meticulously fertilized once prior to sowing.

The annual fertilizer dosing at the cereal field was mineral fertilizer at 666.6 kg ha^{-1} (120 kg N-min ha^{-1}), NEO at 37.6 tons ha^{-1} (120 kg N-min ha^{-1}), organic fertilizer at 41 tons ha^{-1} (65 kg N-min ha^{-1}), and no fertilizer. The grass field annually received mineral fertilizer at a dose of 650 kg ha^{-1} (120 kg N-min ha^{-1}) in the spring and 500 kg ha^{-1} (90 kg N-min ha^{-1}) after the first harvest, NEO at 37.5 tons ha^{-1} (120 kg N-min ha^{-1}) in the spring and 28 tons ha^{-1} (90 kg N-min ha^{-1}) after the first harvest, organic fertilizer at 41 tons ha^{-1} (65 kg N-min ha^{-1}) in spring and 30.5 tons ha^{-1} (50 kg N-min ha^{-1}) after the first harvest, and no fertilizer.

NEO type B had a pH of 5.35 and contained 1480 mg L^{-1} NH_4^+, 777 mg L^{-1} NO_2^-, and 1250 mg L^{-1} NO_3^-, totaling 3507 mg L^{-1} N. The cattle slurry used in this experiment had a pH of 7.32, and it contained 1804 mg L^{-1} NH_4^+ and 149 mg L^{-1} NO_2^-, totaling 1953 mg L^{-1} N.

Mineral fertilizer was administered in pellet form, while NEO and untreated slurry were liquids. Before being bottled or distributed, the containers were thoroughly stirred to dissolve the sediments, ensuring the homogeneity of the liquid fertilizers. The fertilizers were manually applied using containers and quickly mixed with the soil using a tractor before sowing or dispersed on the top of the grass canopy.

The cereal field had a soil texture that was categorized as sandy clay loam, with a pH value of 7.4 and an organic matter content of 4.5%. The available phosphorus content was average (P-AL = 11 mg/100 g), while the available potassium content was below average (K-AL = 5 mg/100 g). The total pore volume was 41.4%, and the field water capacity was 33.6% Volumetric Water Content (VWC). Additionally, the grass field had a soil texture classified as clay loam, consisting of approximately 10% clay. It had a pH value of 5.7 and an organic matter content of 5.1%. The phosphorus and potassium statuses were average (P-AL = 8, K-AL = 7, res). However, the potassium reserve was high ($KHNO_3$ = 140).

The cereal field was sown with spring wheat 'Mirakel' (Graminor, Norway) (220 kg ha^{-1}) in 2021 and barley 'Rødhette' (Graminor, Norway) (180 kg ha^{-1}) in 2020. The grass field was seeded in 2019 with a combination of timothy, meadow fescue, and red clover. The cereal field received two applications of herbicides: Ariane S (Corteva Agriscience, Puerto Rico) in June and Roundup (Bayer, Germany) after the growing season. There was no irrigation. With a comparatively cool May and slightly above-average precipitation of 78 mm in May and 62 mm in June, the 2021 season at Blæstad was reasonable regarding cereal growth (yr.no, 2023). The season was favorable, and precipitation amounts were about average in Trøndelag.

The abundance of springtails was measured in the cereal and grass fields, which were sampled twice in 2021. The first sampling was conducted on June 15, 2021, a few weeks after fertilization, while the second was completed on October 20, 2021, after harvesting. The temperature during the first sampling was 20°C, while it was 6°C during the second sampling, carried out after some rainy days.

Soil sampling involved collecting three diametric samples from each replicate's corners and center of the field plots. A corer (5 cm tall, 5 cm diameter = 98.17 cm^3 volume) was hammered into the surface soil, and the samples were collected into zipper bags using a spade (Schinner et al., 1996) (Figure 8). The samples were immediately transported to the lab and overturned on slightly modified Berlese funnels (Berlese, 1905; Schinner et al., 1996; Tullgren, 1918).

Figure 8: Soil sampling for determining the abundance of springtails in different fertilized plots. Three diametrically samples were collected from each plot replicate.

The samples were exposed to moisture, heat, and light gradients, which caused the springtails to flee the heat source (60 W lamp) (O'Connor, 1962). The organisms then passed through a mesh screen and fell into a collection tube filled with 91% ethanol, allowing us to preserve them for further investigation. The samples were left in extraction units for a week on each sampling occasion (Figure 9). After the extraction, the abundance of springtails in the samples was estimated using a light microscope. The springtail abundances were scaled up to 1 m^2 and − 5 cm depth based on the number of soil cores that could fit into 1 m^2 (169.8 corers).

Figure 9: Experimental setup using Tullgren funnels to extract springtails from soil samples collected at each sampling occasion.

2.3.3. Evaluating changes in abundance and weight of soil macrofauna, e.g., earthworms (*Lumbricus sp.*) (Paper 2)

Earthworms, medium to large-sized oligochaetes, are substrate feeders that play a vital role in soil decomposition, leading to increased soil fertility (Schinner et al., 1996). Their distribution is influenced by environmental factors such as moisture, soil type, pH, and vegetation. Earthworms can be categorized into three ecological groups: litter dwellers, horizontal burrowers, and deep burrowers (Bouché, 1977). They comprise a significant portion of the biomass in loamy meadows but are scarce in shallow or acidic soils (Graff, 1984; Sims, Gerard, London, & Association, 1985).

In our study, we aimed to develop a protocol for assessing the immediate impact of fertilizers on earthworms. We used a mixture of juveniles and adults of the most common Norwegian earthworms - geophagous field worms (*Aporrectodea caliginosa*) and pink worms (*Aporrectodea rosea*) - along with other species found in Norwegian arable soil such as dew worm *Lumbricus terrestris, L. rubellus*, and a few of the less common *Allolobophora chlorotica* (Pommeresche & Løes, 2009). The earthworms used in our trials were collected from an organic vegetable garden near the experimental field.

The experiment was conducted twice, in June 2021 and June 2022, with three replicates each. The study was carried out at Blæstad experimental farm, Innlandet (60°49′11.7″ and N 11°10′48.4″ E). Four fertilizer treatments were used, including (1) no fertilizer, (2) mineral fertilizer (Yara Mila) at a rate of 666 kg per hectare, (3) NEO type B (in 2021) and type D (in 2022) at a rate of 3.4 tons per hectare, and (4) untreated slurry at a rate of 3.7 tons per hectare as an organic fertilizer. The NEO and mineral fertilizers contained almost equal amounts of N-min per hectare in both experiments. Each experiment lasted eight days.

The developed protocol was as follows:

1. Dig holes with a diameter of 30 cm and a depth of 20 cm in the field. Inspect the soil visually to ensure no earthworms are present.
2. Place earthworm-proof but water-permeable textile, previously tested, into the holes.
3. Collect earthworms (*Lumbricidae*) from soil with similar conditions to the experimental field two days before the experiment and store them in a pile of soil until the experiment day. On the experiment day, detach the earthworms from the soil pile, sort them, and weigh them. Choose an equal number of worms with a similar total weight and deposit them in separate containers, marking them accordingly. Do not rinse the worms before weighing them, i.e., handle them carefully and minimize their detachment from the soil (Figure 10).
4. Fill 10 cm of soil back into the holes and place the earthworms on top.
5. Thoroughly mix the fertilizer with the following 5 cm of soil and fill it back into the hole.
6. Spread 100 g of grass over this layer as a food source for the worms.
7. Scatter 2 cm of loose soil over the grass layer.
8. Finally, scatter the last 3 cm of loose soil on top.
9. Gather the outer edges of the textile and close them over the top. Place a heavy substance, such as a stone, on top to prevent wind opening or bird feeding. Cover the experimental units with white plastic tarpaulin on sunny days to avoid excessive temperature caused by the sun and the black textile.

10. At the end of the 8-day experiment, lift the soil bags and disperse them over a flat surface. Next, handpick the living earthworms, count them, and weigh them in less than five minutes to avoid desiccation.

Figure 10: Earthworms separated and weighed before being placed into the designated experimental holes.

2.3.4. Evaluating changes in the soil nitrogen cycle, e.g., nitrification performed by soil nitrifying microbes (Paper 3)

As the ultimate approach, we investigated and compared the effect of NEO on soil nitrification potential with other conventional fertilizers. To determine how NEO affected soil potential nitrification, we employed three experimental setups: 1) fertilized soil from the fields, 2) fertilized soil in the lab incubated as stirred soil slurries, and 3) fertilized soil in the lab incubated as non-agitated loose soil.

Field fertilized soil samples were collected on the same date from the same fields for springtail abundance studies (see section 2.3.2) (Figure 7). First, we sampled ten representatives from each experimental plot to a depth of 20 cm using an 80 mm diameter corer. Then, the representatives for each plot were blended to create a bulk sample in a bucket, and coarse rocks or roots were removed (Figure 11). Finally, we transferred each bulk sample to a plastic zipper bag to prevent desiccation and stored them in a cooling room at 4°C until processing in the laboratory (Öhlinger, Eibelhuber, & Vinzenz, 1993).

Figure 11: A corer was utilized to collect samples from the experimental plots treated with different fertilizers. Ten representative samples were extracted diametrically from each plot replicate.

To prepare the soil samples for laboratory analysis, we sieved them to 3 mm and removed any remaining plant debris to ensure homogeneity. Since the soil moisture was still higher than required for lab analysis, we placed the sieved soil in open zipper bags at room temperature for 24 hours before transferring them back to the refrigerator. The soils were refrigerated until laboratory experiments (Öhlinger et al., 1993) (Figure 12).

Figure 12: To prepare the soil samples for potential nitrification investigations, they were sieved down to 3mm, and the remaining plant debris was removed.

Sieving soil increases soil biological activity by exposing soil fractions to oxygen and making more nutrients available. To recover the original biological activity in the soil, we stored the soil for six days before experimentation (Schinner et al., 1996). Then, to evaluate the gravimetric soil moisture, we followed the protocol of drying (Kellogg Biological, 2019). We estimated the gravimetric water content of the cereal field soil to be 24.9% and the grass field soil to be 33.9% on average.

Next, we meticulously measured and weighed 12.5 grams of soil from different treatments and replicates in the cereal field and 13.5 grams from those in the grass field. This quantity approximated 10 grams of soil when considering its dry weight, factoring in the gravimetric water content. These soil samples were collected from various fertilization treatments and transferred into 120-milliliter serum bottles. We added 50 milliliters of deionized (DI) water to each bottle, ensuring uniform conditions across all samples.

We promptly sealed all the bottles with rubber septa and secured them with aluminum crimp seals. In order to establish a consistent incubation environment, we placed the sealed bottles onto a horizontal shaker and incubated them at room temperature for a duration of 66 hours.

As mentioned, in addition to examining the impact of field fertilization on soil nitrification potential, we conducted experiments using two laboratory fertilization and incubation setups (agitated soil slurries and loosely incubated soil). This allowed us to investigate and compare the immediate effects of different fertilizers on nitrification potential.

First, to eliminate any long-term field treatment confounding factors, we combined soil samples from different treatment plots and created two bulk samples: cereal and grass fields. Then we weighed 12.5 g of cereal field soil and 13.5 g of grass field soil, equal to 10 g of dry soil weight, into 36 serum bottles of size 120 ml each (18 bottles per field).

Six fertilization treatments with three replicates were prepared (Figure 13), including untreated cattle slurry (Raw S), untreated biogas digestate (Raw D), NEO made from cattle slurry (NEO S), NEO made from biogas digestate (NEO D), untreated cattle slurry acidified with HCl (Raw S acidified), and a concentrated NH_4Cl solution (positive control). We adjusted the amount of each fertilizer based on NH_4^+ content (the nitrification's primary substrate), aiming to provide 170 kg NH_4^+-N ha^{-1} for the 5 cm depth soil, translating to 17 g fertilizer m^{-2}, 0.283 mg N g^{-1} dry weight soil, or 2.83 mg NH_4^+-N $bottle^{-1}$.

Figure 13: The lab-fertilized soil samples, incubated as agitated soil slurries, are being prepared to assess the impact of different fertilizers on soil nitrification.

Raw S, Raw D, NEO S, NEO D, and acidified raw S had NH_4^+-N concentrations of 1606 mg L^{-1}, 3020 mg L^{-1}, 1732 mg L^{-1}, 2580 mg L^{-1}, and 1606 mg L^{-1}, respectively. Therefore, we added 1.76 ml raw S, 0.94 ml raw D, 1.64 ml NEO S, 1.10 ml NEO D, and 1.76 ml acidified raw S to the designated bottles, followed by 50 ml of DI H_2O. Next, we mixed the soils with 50 ml of a solution containing 216.5 mg N L^{-1} for the ammonium chloride treatments. We then capped all the bottles using rubber septa and aluminum crimp seals and placed them on a horizontal shaker for incubation at room temperature for 43 hours.

Additionally, to investigate the impact of soil disintegration and shaking on nitrification activity, we conducted an experiment where lab-fertilized soils were loosely incubated for 73 hours. We chose to use soil from the cereal field, as the fertilization effects on the slurried soils were similar for both cereal and grass soils, and the cereal field soil had a higher pH and better buffering capacity than the grass field soil.

We arranged three sets of 15 x 50 ml sterile centrifuge tubes containing 12.5 g of cereal field soil (equivalent to approximately 10 g of dry-weight soil). We then applied five fertilization treatments with three replicates to all three sets simultaneously: raw S, raw D, NEO S, NEO D, and ammonium chloride (Figure 14).

Figure 14: The lab-fertilized soil samples, incubated as loose soil, are being prepared to assess the impact of different fertilizers on soil nitrification.

To ensure consistency, we adjusted the fertilizer amounts to 85 kg NH_4^+-N ha^{-1} (the nitrification's primary substrate), based on a bulk density of 1.2 g cm^{-3} and a soil depth of 5 cm, yielding 8.5 g fertilizer per square meter, or 0.142 mg N per gram soil, equated to 1.42 mg NH_4^+-N per tube. Thus, we applied 0.88 ml of raw S, 0.47 ml of raw D, 0.82 ml of NEO S, and 0.55 ml of NEO D to designated tubes, and we prepared a solution of ammonium chloride by mixing 0.54 g of NH_4Cl in 100 ml of DI H_2O, of which we applied 1 ml to designated tubes. Finally, we capped the tubes and incubated them for 73 hours at room temperature.

To determine the nitrification rates, colorimetric assays and a spectrophotometer (Infinite® F50, TECAN Life Sciences, Männedorf, Switzerland) were used to measure the $NO_3^- + NO_2^-$ accumulation rate. The Greiss reaction assay (Griess, 1858; Keeney & Nelson, 1983; Killham, 1998; Thion & Prosser, 2014; Z. Wang, 2010) was used to measure nitrite concentration (NO_2^-). The nitrite + nitrate ($NO_3^- + NO_2^-$) concentration was determined using the assay adapted from (Doane & Horwáth, 2011), using Vanadium (III) chloride (VCl_3) to oxidize nitrite to nitrate.

The potential nitrification rates of field-fertilized soil were estimated by plotting the $NO_3^- + NO_2^-$ accumulation in soil slurries during incubation using polynomial regression. The soil slurries were sampled four times at 0, 19, 44.5, and 66 h into the incubation, with 1 ml of the liquid extracted using a syringe and transferred to 2 ml Eppendorf tubes (Figure 15). The extracted subsamples were centrifuged at 10000 rpm and 4°C for 10 minutes before determining NO_2^- and $NO_3^- + NO_2^-$ concentrations as described above. In addition, soil pH was screened before and after the experiment using a handheld pH meter (Hach- H-Series H160, Loveland, CO, USA) equipped with an ISFET sensor (Mettler Toledo, Stockholm, Sweden) due to the pH sensitivity of nitrification.

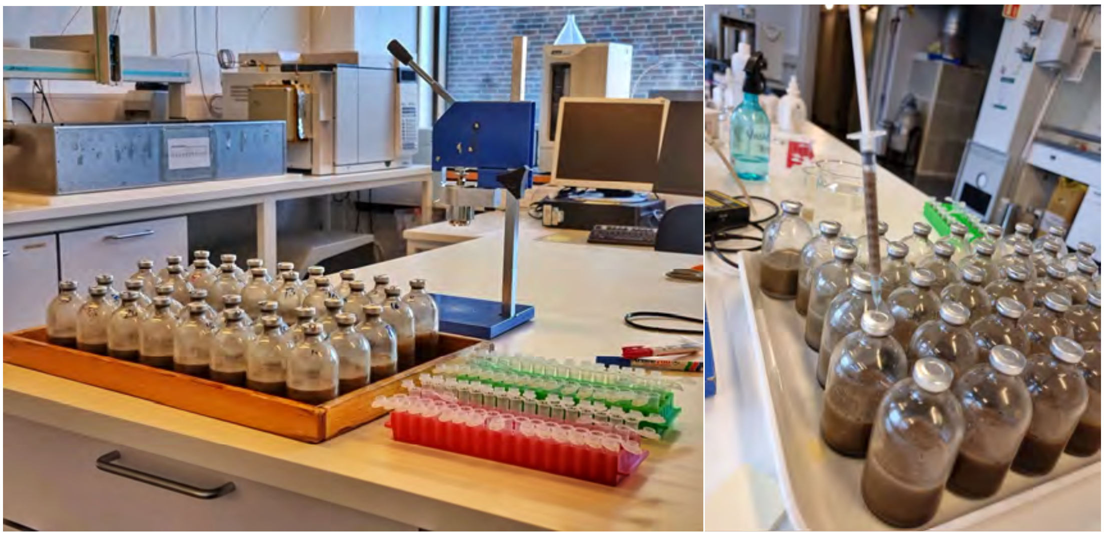


Figure 15: The soil slurries were sampled at different time points after the incubation, with 1 ml of the liquid extracted using a syringe and transferred to 2 ml Eppendorf tubes.

Also, lab-fertilized soil incubated as soil slurries followed the abovementioned procedure (Figure 15), except for three extraction rounds at 0, 19, and 43 hours after incubation. The extracts had to be diluted ten times with DI H_2O after the first extraction round and 20 times after the second and third extraction due to the high nitrate contents coming along with the NEO fertilizers. Soil pH was screened before and after incubation for all samples.

For lab fertilized soil incubated as non-agitated loose soil, $NO_3^- + NO_2^-$ accumulation over time was measured by sacrificing each of three tubes per treatment 1, 25, and 73 h into the incubation. $NO_3^- + NO_2^-$ was extracted by adding 30 ml 2 M KCL and shaking for one hour, then using 1 ml of the supernatant for $NO_3^- + NO_2^-$ analysis as described above.

2.3.5. Evaluating the NEO's impact on crop yields (Paper 1 and Paper 4)

To assess and compare the impact of NEO on crop yields against conventional fertilizers, we conducted experiments in both controlled (**Paper 1**) and field conditions (**Paper 4**). Under controlled conditions, we assessed the performance of grasses within a growth chamber, while our field experiments were centered on cereals. For a comprehensive description of the experimental setup under controlled conditions, see section 2.3.1.

The field studies' experimental setup involved a randomized complete block design with four replicates, encompassed Series 1 and Series 2. In Series 1, the fertilizer plots measured 10×3 m, with a harvested area of 1.5×8.5 m within each plot. In Series 2, the fertilizer plots were smaller, measuring 2.5×8 m, with harvest plots at 1.5×6.5 m. The reduced harvesting area than the plot area was to mitigate the marginal impacts of fertilization on our crop yields.

The field trials took place over three years in Norway's four indicative regions known for cereal production: Tønsberg, Årnes, Hamar, and Stjørdal. Elaborated information concerning these locations and their corresponding soil types can be found in Table 1:

Table 1: The trial numbers, location coordinates, and soil quality information at the trial sites. The barley and wheat were all spring-sown types.

Series	Trial	Location	Crops, varieties, and years	Detailed location and coordinates	Soil type and key soil parameters
1	1	3	Barley' Salome' 2020, Wheat 'Betong' 2021, Barley' Bente' 2022	3 km east of Hamar (60,81830°N, 011,17968°E)	Loam, 4,5 % organic, pH 7,4
1	2	3	Wheat 'Mirakel' 2020, Barley' Anita' 2021, Wheat 'Betong' 2022	3 km east of Hamar (60,81830°N, 011,17968°E)	Loam, 4,5 % organic, pH 7,4
1	3	2	Wheat 'Helmi' 2021	3 km west of Årnes (60,12604°N, 11,39471°E)	Silt loam, 4,0 % organic, pH 6,0
1	4	2	Barley 'Brage' 2021	3 km west of Årnes (60,12604°N, 11,39471°E)	Silt loam, 4,0 % organic, pH 6,0
2	5	1	Wheat 'Betong' 2021	5 km west of Tønsberg (59.294937°N, 10.318813°E)	Silt loam, 6,5 % organic, pH 6,2
2	5	1	Wheat 'Betong' 2022	15 km north of Tønsberg (59.384537°N, 10.232651°E)	Silt loam, 4,8 % organic, pH 6,9
2	6	4	Barley 'Thermus' 2021	4 km north of Stjørdal (7041109°N, 593647°E)	Loam, 2,7 % organic, pH 6,1
2	6	4	Barley 'Thermus' 2022	4 km north of Stjørdal (7037496°N 597733°E)	Loam, 2,7 % organic, pH 6,1

The fertilizers employed in our trials were: 1. Untreated slurry, sourced from the Norwegian University of Life Sciences farm and made from cattle slurry; 2. NEO (Nitrogen Enriched Organic Fertilizer), Derived from the same slurry as the "Untreated slurry" but processed through the N2 Applied unit. NEO contains approximately 50% ammonium, 30% nitrate, and 20% nitrite, reducing its pH to around 5.2; the nitrate and nitrite levels vary significantly yearly; 3. Mineral Fertilizer 18-3-15: A commercially available mineral fertilizer manufactured by Yara. It contains 18% N, 3% phosphorus (P), and 15% potassium (K). The N in this fertilizer comprises slightly more ammonium than nitrate. This choice was made due to the similarity in plant-available nutrients to NEO; 4. Mineral Fertilizer Opti-NS (27-0-0): This fertilizer is a combination of N and sulfur (S) at a ratio of 27-0-0. The N content in Opti-NS consists of equal amounts of ammonium and nitrate.

Our main target was to evaluate the impact of NEO on crop yield compared to other alternatives used by farmers. To establish a benchmark, we set a target of 120 kg N-min ha^{-1}

for both wheat and barley, considering it a typical level for barley in Norway's grain regions, though slightly lower than the common usage in spring wheat.

The treatments and their labels used in our trials are as follows:

In Series 1 for the year 2020, the treatments were:

- MaF51: 51 kg N-min ha^{-1} in Filtered untreated slurry.
- Ma56: 56 kg N-min ha^{-1} in untreated slurry.
- NEO102: 102 kg N-min ha^{-1} in NEO.
- MiNEO 104: 12 kg N-min ha^{-1} in mineral fertilizer 18-3-15 applied to the trial plots before sowing, combined with 92 kg N-min ha^{-1} in NEO at Zadoks GS13 (three leaves stage).
- Mi51: 51 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi91: 91 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi123: 123 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- MaMi123: 56 kg N-min ha^{-1} in untreated slurry, combined with 67 kg N-min ha^{-1} in mineral fertilizer Opti-NS.

For Series 1 in both 2021 and 2022, the treatments were:

- Ma65: 65 kg N-min ha^{-1} in untreated slurry (manure).
- NEO120: 120 kg N-min ha^{-1} in NEO.
- MiNEO 120: 12 kg N-min ha^{-1} in mineral fertilizer 18-3-15 applied to the trial plots before sowing, along with 108 kg N-min ha^{-1} in NEO at Zadoks GS13 (three leaves stage).
- Mi65: 65 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi91: 91 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi120: 120 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- MaMi120: 65 kg N-min ha^{-1} in untreated slurry combined with 55 kg N-min ha^{-1} in mineral fertilizer Opti-NS.
- NoF: No fertilizer.

In Series 2 for both 2021 and 2022, the treatments were:

- NEO120: 120 kg N-min ha^{-1} in NEO.
- Ma65: 65 kg N-min ha^{-1} in untreated slurry.

- MaMi120: 65 kg N-min ha^{-1} in untreated slurry and 55 kg N-min ha^{-1} in mineral fertilizer Opti-NS.
- Mi30: 30 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi55: 55 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi80: 80 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi105: 105 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- Mi120: 120 kg N-min ha^{-1} in mineral fertilizer 18-3-15.
- NoF: No fertilizer.

All fertilization treatments were administered on the day of sowing except for the MiNEO120 treatment. For this treatment, we applied 12 kg N-min ha^{-1} of mineral fertilizer Yara Mila complete fertilizer 18-3-15 to the trial plots before sowing. Additionally, 108 kg N-min ha^{-1} of NEO was applied at the three-leaf stage Zadoks GS13 (Zadoks, Chang, & Konzak, 1974). The fertilization involved spreading the fertilizers onto the trial plots and incorporating them into the soil using a disc harrow or spreading them manually on plants. The sowing of grains occurred shortly after the fertilization process.

3. Main findings

3.1. Paper 1

The main objective of **Paper 1** was to assess the impact of the novel Nitrogen-Enriched Organic fertilizer (NEO) on Italian ryegrass (*Lolium multiflorum Lam.*) yield and soil fauna feeding activity, as well as to investigate any potential correlation between the two.

3.1.1. Dry matter (DM) yields

In trial one, the dry matter yield was influenced by the N content provided through fertilization, resulting in a significant difference ($p = 0.001$) among the various fertilizing treatments. Linking the DM yields from the first and second harvests, it was observed that the highest amount of mineral fertilizer (235 kg N-min ha^{-1}) yielded between 12% and 46% more than other fertilization treatments and 81% more compared to no fertilizer. Notably, NEO with 175 kg N-min ha^{-1} yielded higher than untreated manure (73 kg N-min ha^{-1}) and was comparable to mineral fertilizer with the same N-min content as in NEO (175 kg N-min ha^{-1}). Conversely, the lowest DM yields were obtained with no fertilizer (Table 2A).

Like trial one, trial two demonstrated a significant effect of the N amount in the fertilizer on DM yields ($p = 0.001$). Mineral fertilizer with 175 kg N-min ha^{-1} and 155 kg N-min ha^{-1} resulted in higher DM yields than other treatments. Specifically, mineral fertilizer containing 175 kg N-min ha^{-1} resulted in a yield increase ranging from 3% to 63% compared to other fertilization treatments and 81% higher yield than no fertilizer. The different NEO fertilizers, all with 175 kg N-min ha^{-1}, yielded within the same range as mineral fertilizers with 115–155 kg N-min ha^{-1}. On the other hand, DM yields were lower in mineral fertilizer with 80 kg N-min ha^{-1}, organic fertilizer with 73 kg N-min ha^{-1}, and mineral fertilizer with 60 kg N-min ha^{-1} treatments compared to the other treatments. The lowest DM yields were observed in no fertilizer treatment among all the treatments (Table 2B).

3.1.2. Soil fauna feeding activity

In trial one, soil fauna feeding activity was evaluated for both early and late fertilization effects (Figure 16). Regarding the early effects, a significant difference ($p = 0.001$) was observed among the different fertilizing treatments. Mineral fertilizer with 205 kg N-min ha^{-1} exhibited higher soil fauna feeding activity than mineral fertilizer with 115 kg N-min ha^{-1}, NEO type B with 175.4 kg N-min ha^{-1}, and NEO type C with 175.4 kg N-min ha^{-1} (Table 2C).

Figure 16: Examples of Bait-lamina strips with consumed bait material; each empty hole represents 6.25% soil fauna feeding activity.

Similarly, in trial two, significant differences ($p = 0.001$) were observed among the different fertilizing treatments concerning early effects. No fertilizer, NEO type B with 175.4 kg N-min ha^{-1}, and mineral fertilizer with 60 kg N-min ha^{-1} showed higher soil fauna feeding activity than other treatments. However, 175 kg N-min ha^{-1} of mineral fertilizer exhibited the lowest soil fauna feeding activity (Table 2D).

For the late effects of fertilizers on soil fauna feeding activity in trial one, there was a significant difference ($p = 0.001$) among the different fertilizing treatments similar to the early effects. Mineral fertilizer with 205 kg N-min ha^{-1} resulted in higher soil fauna feeding activity than organic fertilizer with 73 kg N-min ha^{-1}, all types of NEO with 175.4 kg N-min ha^{-1}, and all combinations of organic fertilizer + mineral fertilizer. NEO type A with 175.4 kg N-min ha^{-1} and organic fertilizer + mineral fertilizer with 205 kg N-min ha^{-1} exhibited the lowest soil fauna feeding activity (Table 2E).

Furthermore, a consistent pattern was observed, where the highest feeding activity occurred below 5 cm from the soil surface. In trial one, the feeding activity was lowest at a depth of 2 cm ($p = 0.001$) (Table 2F). In trial two, although the differences were insignificant ($p = 0.08$), the same pattern of feeding activity at different depths was observed during the early effects after fertilization (Table 2G). The late fertilization effects followed a similar pattern, but the differences were not statistically significant ($p = 0.37$) (Table 2H).

In general, no correlation was observed between dry matter yields and the soil fauna feeding activity, and NEO fertilizers did not negatively impact feeding activity more than the other fertilizers.

Table 2: Effects of different fertilization treatments on ryegrass dry matter yields (g pot^{-1}) in two trials (A, B) and soil fauna feeding activity (%) in different evaluation time points and depths (C-H). Games-Howell pairwise comparison method at a 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

A: Ryegrass dry matter yields in trial one	**Yield (g)**	**Group**
Mineral fertilizer 235 kg N ha-1	16.9	A
Mineral fertilizer 175 kg N ha-1	14.9	AB
NEO type B 175.4 kg N ha-1	14.2	AB
NEO type C 175.4 kg N ha-1	14.1	AB
Mineral fertilizer 205 kg N ha-1	14	AB
Organic fertilizer + MF 175 kg N ha-1	13.4	AB
Organic fertilizer + MF 205 kg N ha-1	13.3	AB
NEO type A 175.4 kg N ha-1	13.1	AB
Mineral fertilizer 145 kg N ha-1	12.8	AB
Organic fertilizer + MF 145 kg N ha-1	12.7	AB
Organic fertilizer + MF 115 kg N ha-1	11.9	B
Mineral fertilizer 115 kg N ha-1	11.6	B
Organic fertilizer 73 kg N ha-1	9.1	B
No fertilizer	3.2	C
B: Ryegrass dry matter yields in trial two	**Yield (g)**	**Group**
Mineral fertilizer 175 kg N ha-1	14.1	A
Mineral fertilizer 155 kg N ha-1	13.8	A
NEO type A 175.4 kg N ha-1	13.4	A
NEO type C 175.4 kg N ha-1	13.2	A
Mineral fertilizer 135 kg N ha-1	13.1	A
Mineral fertilizer 115 kg N ha-1	12.4	A
NEO type B 175.4 kg N ha-1	11.9	AB
Mineral fertilizer 80 kg N ha-1	10.6	B
Organic fertilizer 73 kg N ha-1	10.3	BC
Mineral fertilizer 60 kg N ha-1	9.1	C
No fertilizer	2.7	D
C: Soil fauna feeding activity, early effects in trial one	**Feeding activity (%)**	**Group**
Mineral fertilizer 205 kg N ha-1	48.4	A
Mineral fertilizer 175 kg N ha-1	46	AB
No fertilizer	40.6	AB
Organic fertilizer 73 kg N ha-1	39.1	AB
Organic fertilizer + MF 205 kg N ha-1	37.2	AB
Organic fertilizer + MF 145 kg N ha-1	34.6	AB
Mineral fertilizer 145 kg N ha-1	33.1	AB
Organic fertilizer + MF 115 kg N ha-1	32.2	AB
Mineral fertilizer 235 kg N ha-1	28.1	AB
Organic fertilizer + MF 175 kg N ha-1	25.2	AB
NEO type A 175.4 kg N ha-1	22.6	AB
Mineral fertilizer 115 kg N ha-1	21.6	B
NEO type B 175.4 kg N ha-1	20.3	B
NEO type C 175.4 kg N ha-1	19.2	B
D: Soil fauna feeding activity, early effects in trial two	**Feeding activity (%)**	**Group**
No fertilizer	48.7	A
Mineral fertilizer 60 kg N ha-1	48.7	A
NEO type B 175 kg N ha-1	46.3	A
NEO type A 175 kg N ha-1	42.7	AB
Mineral fertilizer 80 kg N ha-1	41.9	AB
Mineral fertilizer 135 kg N ha-1	40.1	AB
NEO type C 175 kg N ha-1	38.8	AB

Organic fertilizer 73 kg N ha-1	37.5	AB
Mineral fertilizer 115 kg N ha-1	36.7	AB
Mineral fertilizer 155 kg N ha-1	26.8	AB
Mineral fertilizer 175 kg N ha-1	13.8	B
E: Soil fauna feeding activity, late effects in trial one	**Feeding activity (%)**	**Group**
Mineral fertilizer 205 kg N ha-1	69.7	A
Mineral fertilizer 175 kg N ha-1	69	AB
No fertilizer	68.7	ABC
Mineral fertilizer 145 kg N ha-1	59.9	ABCD
Mineral fertilizer 115 kg N ha-1	55.4	ABCD
Mineral fertilizer 235 kg N ha-1	53.1	ABCD
NEO type C 175.4 kg N ha-1	42.1	BCD
NEO type B 175.4 kg N ha-1	41.9	BCD
Organic fertilizer + MF 115 kg N ha-1	41.4	BCD
Organic fertilizer + MF 175 kg N ha-1	41.4	BCD
Organic fertilizer 73 kg N ha-1	36.7	CD
Organic fertilizer + MF 145 kg N ha-1	35.9	CD
NEO type A 175.4 kg N ha-1	34.3	D
Organic fertilizer + MF 205 kg N ha-1	30.1	D
F: Depth of feeding activity, early effects in trial one	**Feeding activity (%)**	**Group**
8 cm	43	A
6.5 cm	39	AB
7.5 cm	37	ABC
7 cm	36	ABC
5.5 cm	35	ABC
6 cm	32	ABC
0.5 cm	31	ABC
1 cm	31	ABC
3 cm	30	ABC
1.5 cm	30	ABC
5 cm	29	ABC
3.5 cm	29	ABC
2.5 cm	29	ABC
4 cm	26	BC
4.5 cm	25	BC
2 cm	23	C
G: Depth of feeding activity, early effects in trial two	**Feeding activity (%)**	**Group**
5.5 cm	43	A
7 cm	42	A
4.5 cm	42	A
6 cm	41	A
8 cm	41	A
6.5 cm	41	A
4 cm	40	A
5 cm	39	A
7.5 cm	38	A
0.5 cm	38	A
3.5 cm	37	A
3 cm	37	A
2.5 cm	36	A
1 cm	36	A
2 cm	28	A
1.5 cm	28	A
H: Depth of feeding activity, late effects in trial one	**Feeding activity (%)**	**Group**
7 cm	55	A
8 cm	53	A
6.5 cm	53	A
7.5 cm	51	A
6 cm	51	A
2.5 cm	48	A

5 cm	48	A
3.5 cm	48	A
4.5 cm	47	A
1 cm	47	A
5.5 cm	46	A
1.5 cm	46	A
0.5 cm	45	A
3 cm	45	A
2 cm	44	A
4 cm	43	A

3.2. Paper 2

According to N2 Applied, NEO can serve as a sustainable replacement for traditional fertilizers used in agriculture. Nonetheless, the impact of this product on soil-dwelling organisms needs to be elucidated. Therefore, **Paper 2** aimed to investigate and compare the effects of NEO on the changes in soil fauna feeding activity 7, 14, and 21 weeks after fertilization, as well as the abundance of springtails and earthworms.

3.2.1. Soil fauna feeding activity

To evaluate the influence of different fertilization treatments on soil fauna feeding activity, we conducted a growth chamber experiment where all variables, except fertilization, were kept constant. We examined the early effects (seven weeks), mid-term effects (14 weeks), and late effects (21 weeks).

A significant early fertilization effect on the feeding activity was observed ($p = 0.001$). Nevertheless, this effect was attributed to the quantity of fertilizer applied rather than the specific fertilizer type. After seven weeks, soil fauna feeding activity was increased with mineral fertilizer 73 kg N-min ha^{-1}, organic fertilizer 73 kg N-min ha^{-1}, NEO type D 73 kg N-min ha^{-1}, and organic + mineral fertilizer 175 kg N-min ha^{-1} compared to no fertilizer. NEO type D 175 kg N-min ha^{-1} and mineral fertilizer 175 kg N-min ha^{-1} showed lower soil fauna feeding activity than unfertilized soil. Notably, the mixture of organic and mineral fertilizers was the only high N content treatment that significantly improved soil fauna feeding activity compared to no fertilizer (Table 3A).

Table 3: Effects of different fertilization treatments on soil fauna feeding activity (%) in different evaluation time points (C-H), springtail abundance across different sampling points and locations (M-P), abundance and weight change (g per experimental plot) of earthworms (Q-T). Games-Howell pairwise comparison method at a 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

A: Soil fauna feeding activity (early effects, 7 weeks), trial three	**Feeding activity (%)**	**Group**
Mineral fertilizer 73 kg N ha-1	78.13	A
Organic fertilizer 73 kg N ha-1	74.74	A
NEO type D 73 kg N ha-1	73.7	AB

Organic fertilizer + MF 175 kg N ha-1	61.98	ABC
No fertilizer	54.17	BC
NEO type D 175 kg N ha-1	49.22	C
Mineral fertilizer 175 kg N ha-1	46.35	C
B: Soil fauna feeding activity (mid-term effects, 14 weeks), trial three	**Feeding activity (%)**	**Group**
Mineral fertilizer 73 kg N ha-1	58.44	A
NEO type D 73 kg N ha-1	55.99	A
Organic fertilizer + Mineral fertilizer 175 kg N ha-1	49.48	A
Organic fertilizer 73 kg N ha-1	48.96	A
NEO type D 175 kg N ha-1	45.57	A
No fertilizer	41.41	A
Mineral fertilizer 175 kg N ha-1	34.38	A
C: Soil fauna feeding activity (late effects, 21 weeks), trial three	**Feeding activity (%)**	**Group**
NEO type D 73 kg N ha-1	49.74	A
Mineral fertilizer 73 kg N ha-1	49.48	A
Organic fertilizer 73 kg N ha-1	46.35	A
No fertilizer	46.09	A
NEO type D 175 kg N ha-1	45.83	A
Mineral fertilizer 175 kg N ha-1	45.57	A
Organic fertilizer + Mineral fertilizer 175 kg N ha-1	45.05	A
D: Soil fauna feeding activity all treatments (early, mid-term, and late effects averages), trial three	**Feeding activity (%)**	**Group**
7 weeks	62.61	A
14 weeks	47.75	A
21 weeks	46.88	A
E: Springtail abundance summer sampling crop field	**Abundance per m^2**	**Group**
Organic fertilizer	509.4	A
NEO	467	A
Mineral fertilizer	297.1	A
F: Springtail abundance summer sampling grass field	**Abundance per m^2**	**Group**
Organic fertilizer	1528	A
NEO	807	A
Mineral fertilizer	467	A
G: Springtail abundance fall sampling crop field	**Abundance per m^2**	**Group**
Organic fertilizer	212.3	A
No fertilizer	212.3	A
NEO	169.8	A
Mineral fertilizer	169.8	A
H: Springtail abundance fall sampling crop field	**Abundance per m^2**	**Group**
Organic fertilizer	1486	A
No fertilizer	1486	A
NEO	1401	A
Mineral fertilizer	1316	A
I: Earthworm abundance change June 2021	**Abundance change plot^{-1}**	**Group**
Organic fertilizer	4.33	A
Mineral fertilizer	4	A
NEO	2.33	A
No fertilizer	-1	A
J: Earthworm weight change June 2021	**Weight change (g) plot^{-1}**	**Group**
Mineral fertilizer	4.07	A
Organic fertilizer	2.87	A
NEO	1.93	A
No fertilizer	-1.67	A
K: Earthworm abundance change June 2022	**Abundance change plot^{-1}**	**Group**
Organic fertilizer	7	A
NEO	7	A
No fertilizer	6	A
Mineral fertilizer	3.67	A
L: Earthworm weight change June 2022	**Weight change (g) plot^{-1}**	**Group**

No fertilizer	0.5	A
Organic fertilizer	0.22	A
NEO	0.05	A
Mineral fertilizer	- 0.95	A

Regarding the mid-term effect at 14 weeks after fertilization, the initial differences in soil faunal feeding activity between treatments converged and became more consistent. However, the reduction was more noticeable among treatments with higher feeding activity in the early weeks. The average feeding activity decreased significantly ($p = 0.001$) from 62.61% at seven weeks to 47.75% at the mid-term evaluation (Table 3D).

Furthermore, there were no significant differences ($p = 0.08$) in feeding activities among the fertilization treatments at the mid-term evaluation. However, mineral fertilizer 73 kg N-min ha^{-1}, NEO type D 73 kg N-min ha^{-1}, organic + mineral fertilizer 175 kg N-min ha^{-1}, organic fertilizer 73 kg N-min ha^{-1}, and NEO type D 175 kg N-min ha^{-1} exhibited higher soil faunal feeding activity compared to no fertilizer. Mineral fertilizer 175 kg N-min ha^{-1} showed the lowest feeding activity (Table 3B).

The late fertilization effect on feeding activity was similar to the mid-term effect, with a slight, insignificant average reduction from the mid-term to the late effect among all fertilization treatments (Table 3D).

Regardless of the type, lower amounts of fertilizer supported higher feeding activity. NEO type D 73 kg N-min ha^{-1}, mineral fertilizer 73 kg N-min ha^{-1}, and organic fertilizer 73 kg N-min ha^{-1} exhibited higher feeding activity than no fertilizer. However, higher fertilizer amounts, NEO type D 175 kg N-min ha^{-1}, mineral fertilizer 175 kg N-min ha^{-1}, and organic + mineral fertilizer 175 kg N-min ha^{-1}, resulted in lower feeding activity (Table 3C). Nonetheless, the difference in feeding activities between the highest and lowest amounts was a maximum of 4.2% and was not statistically significant.

In summary, lower quantities of NEO type D, mineral fertilizer, organic fertilizer, and to some extent, the combination of organic and mineral fertilizer seemed to enhance soil fauna feeding activity in the early weeks following fertilization. However, this initial effect gradually diminished over time, while other treatments, including the no fertilizer, exhibited relatively stable soil fauna feeding activity throughout the experiment.

3.2.2. The abundance of springtails

We investigated and compared the effects of different fertilization treatments on the abundance of springtails in two field locations, one under cereal cultivation and the other under grass cultivation. Both fields were fertilized for two consecutive years, and samplings were conducted before fertilization in early summer and during the fall.

During summer, the abundance of springtails in the cereal field was slightly higher for organic fertilizer than NEO and mineral fertilizer (Table 3E). However, the difference was not statistically significant ($p = 0.25$). The same pattern was observed during the fall, with slightly higher numbers of springtails for organic fertilizer and no fertilizer than NEO and mineral fertilizer (Table 3G, Figure 17). Again, the difference between the fertilization treatments in the fall sampling was not statistically significant ($p = 0.669$).

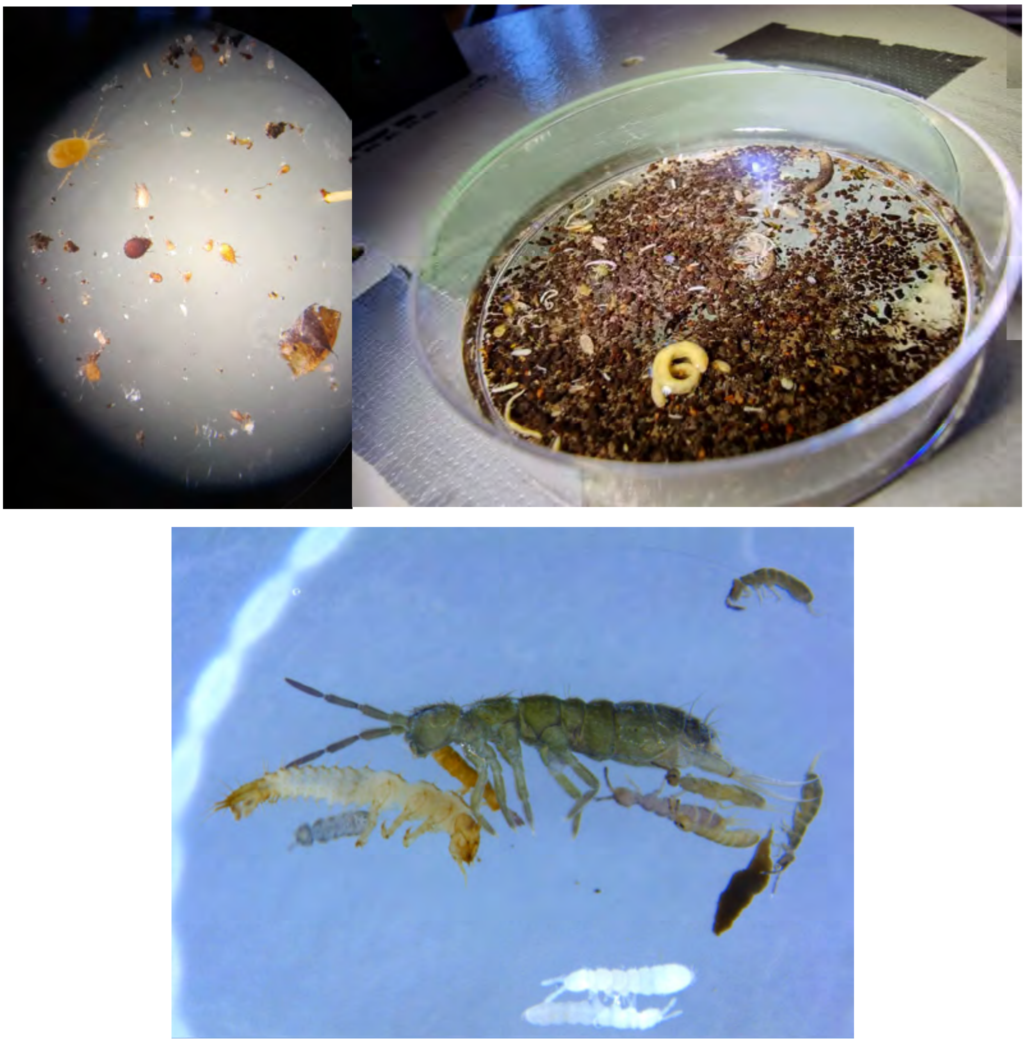


Figure 17: Assessing the abundance of springtails in the extracted samples from different field fertilized plots.

Similarly, no significant fertilization effects on springtail abundance were found in the grass field during summer and fall ($p = 0.404$, $p = 0.943$). However, organic fertilizer showed a higher springtail abundance during summer than NEO and mineral fertilizer (Table 3F). In the fall, slightly higher abundances were observed for organic fertilizer and no fertilizer than NEO and mineral fertilizer (Table 3H).

In general, springtail abundance was higher in the grass field during the fall compared to summer and remained almost identical in the cereal field throughout both seasons. However, none of the fertilizer treatments significantly affected springtail abundance, regardless of the field and season.

3.2.3. The abundance and weight of earthworms

In the 2021 trial, the abundance of earthworms increased for all treatments after eight days, except for the no fertilizer treatment. However, the difference in abundance among the fertilization treatments was not statistically significant ($p = 0.38$) (Table 3I, Figure 18). Further, a similar pattern was observed for the average weight change among all captured earthworms (Table 3J). Similarly, the weight change differences among fertilization treatments were insignificant ($p = 0.27$).

Figure 18: Assessing the weight and number of earthworms eight days after fertilizing the plots (immediate effect)

In the 2022 trial, earthworm abundance increased across all treatments compared to the initial count (Table 3K). However, the differences were not statistically significant ($p = 0.56$). Similar trends were observed in the weight change of captured earthworms, except for mineral fertilizer treatment, with no fertilizer showing a higher weight increment than organic fertilizer and NEO treatments (Table 3L). Nevertheless, the differences in weight change were minimal and inconsequential ($p = 0.33$).

Overall, the outcomes suggest that the various fertilization treatments, including NEO, had no notable impact on earthworm abundance and weight change.

3.3. Paper 3

The elevated acidity and nitrite (NO_2^-) levels found in NEO raise hypothetical concerns due to their potential to generate hazardous inorganic or organic N compounds. Consequently, **Paper**

3 aims to examine and contrast the effects of NEO on soil nitrification with those of conventional fertilizers. Soil nitrification refers to the process wherein aerobic bacteria and archaea convert NH_4^+ into NO_2^- and NO_3^-.

3.3.1. Field-fertilized soil

The nitrification potentials in cereal or grassland field soil samples subjected to different fertilization treatments were not significantly influenced ($p = 0.98$ and $p = 0.68$ in the cereal and grass fields, respectively).

In the cereal field, the control treatment (no fertilizer) had a slightly higher, but statistically non-significant, (NO_2^- + NO_3^-)-N accumulation rate compared to soils that received mineral fertilizer, organic fertilizer, and NEO (Table 4A). In the grassland experiment, organically fertilized soil had a slightly higher nitrification rate than soil that received mineral fertilizer, no fertilizer, or NEO (Table 4B). However, these differences were negligible and insignificant. Remarkably, the nitrification rate was generally lower in the grass field compared to the cereal field (Figure 19).

Table 4: Effects of different fertilization treatments on soil potential nitrification rates (µg (NO2- + NO3-)-N accumulation g DW soil^{-1} day^{-1}) in different experiment setups (A-E). Games-Howell pairwise comparison method at a 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

A: Field-fertilized soil (Cereal field)	**µg (NO_{2-} + NO_3^-)-N g DW soil^{-1} day^{-1}**	**Group**
No fertilizer	32.84	A
Mineral fertilizer	31.41	A
Organic fertilizer	31.37	A
NEO	31.16	A
B: Field-fertilized soil (Grass field)	**µg (NO_{2-} + NO_{3-})-N g DW soil^{-1} day^{-1}**	**Group**
Organic fertilizer	19.25	A
Mineral fertilizer	18.08	A
No fertilizer	17.44	A
NEO	15.94	A
C: Lab-fertilized soil incubated as agitated soil slurries (Cereal field)	**µg (NO_{2-} + NO_{3-})-N g DW soil^{-1} day^{-1}**	**Group**
NEO D	257.37	A
NEO S	108.49	B
Ammonium Chloride	54.016	C
Raw D	46.52	C
Raw S acidified	41.89	**C**
Raw S	34.98	C
D: Lab-fertilized soil incubated as agitated soil slurries (Grass field)	**µg (NO_{2-} + NO_{3-})-N g DW soil^{-1} day^{-1}**	**Group**
NEO D	253.751	A
NEO S	123.788	B
Ammonium Chloride	28.770	C
Raw D	20.327	C
Raw S	17.713	**C**
Raw S acidified	12.174	C
E: Lab-fertilized soil incubated as non-agitated, loosely placed soil	**µg (NO_{2-} + NO_{3-})-N g DW soil^{-1} day^{-1}**	**Group**
NEO D	60.1540	A
Ammonium Chloride	24.5337	B
NEO S	20.5479	B
Raw D	12.9446	BC
Raw S	-1.5813	**C**

Figure 19: The nitrification (NO_2^- + NO_3^--N accumulation rate) was assessed at different time points after incubation and calculated by plotting the NO_3^- + NO_2^- accumulation in the samples during incubation using a polynomial regression.

The native pH in cereal and grass field soils was 7.4 and 5.7, respectively. At the beginning of the incubation, the average pH of fertilized cereal soils (n=4) varied between approximately 6.7 and 7.1, among different treatments. However, the pH of fertilized grass field soils (n=4) varied between 5.4-5.6. The pH slightly decreased during the 66-hour incubation, whereas the grass field samples' pH decreased more than the cereal field samples.

3.3.2. Lab-fertilized soil incubated as agitated soil slurries

Measuring nitrification potentials in agitated soil slurries immediately after amending with different fertilizers indicated significant differences in nitrification rates between fertilization treatments in cereal and grass field soils ($p \leq 0.001$ and $p \leq 0.001$, respectively).

In cereal field soil, NEO made from biogas digestate (NEO D) exhibited the highest nitrification rates, with average values of approximately 250 µg N g DW soil^{-1} day^{-1}, followed by NEO S, indicating a higher (NO_2^- + NO_3^-)-N accumulation rate compared to ammonium chloride, untreated biogas digestate (Raw D), acidified untreated slurry (Raw S acidified), and untreated slurry (Raw S) (Table 4C).

Similarly, within treatments in grass field soil, NEO D and NEO S showed a higher nitrification rate compared to ammonium chloride, raw D, raw S, and raw S acidified (Table 4D). Except for NEO D and NEO S, which indicated identical nitrification rates in cereal and grass field soils, nitrification rates were lower in the grass field soil compared to the cereal field soil.

Furthermore, the pH variations were less evident throughout the 43-hour incubation than in the previous (66-hour incubation) experiment. Within the cereal field samples, only raw S and raw D exhibited a slight pH reduction, whereas other treatments had a similar pH to the beginning. Also, within grass field samples, NEO S, NEO D, raw S acidified, and ammonium chloride slightly increased average pH, whereas raw S and raw D had relatively the same pH as the start.

3.3.3. Lab-fertilized soil loosely placed

When incubating freshly amended soil without agitation, significant differences in $NO_2^- + NO_3^-$ accumulation were observed between fertilization treatments ($p \leq 0.001$).

Similar to the slurried soil experiment, NEO D showed the highest nitrification rate, significantly higher than ammonium chloride and NEO S. Nevertheless, the lowest average nitrification rates were observed in the case of Raw D and Raw S (Table 4E).

3.4. Paper 4

3.4.1. Barley and wheat grain and nitrogen yield – series 1

Exploring barley grain and N yield in Series 1, 2020 indicated that NEO102 yielded on par with MiNEO104, Mi91, and MaMi123. Notably, Mi123 displayed a significantly higher yield compared to all other treatments. In addition, MaF51 exhibited a significantly higher yield than Ma56 (Figure 20).

Turning to wheat grain yield, it was evident that NEO102 outperformed MiNEO104 while maintaining similar yields to Mi91 and MaMi123. Once again, Mi123 stood out with a significantly higher yield than the other treatments. Likewise, MaF51 achieved a higher yield than Ma56 (Figure 20).

The N yield for both barley and wheat followed a similar pattern to the grain yield, albeit with more differences emerging between the treatments (Figure 20).

In terms of barley grain yield in Series One for 2021 and 2022 across the treatments, Mi120 emerged as the lead, outperforming MiNEO120, NEO120, and MaMi120 by margins of 586 kg ha^{-1}, 610 kg ha^{-1}, and 793 kg ha^{-1}, respectively (Figure 20). Notably, both MiNEO120 and NEO120 displayed comparable yields, significantly surpassing Ma65 and aligning within the range of Mi91.

Regarding the wheat grain yield, in contrast to the barley findings, MiNEO120 yielded slightly higher than other treatments, with insignificant differences between Mi120, NEO120, and MaMi120 (Figure 20). While NEO120's yield fell within the range of Mi91, it still significantly outperformed Ma65.

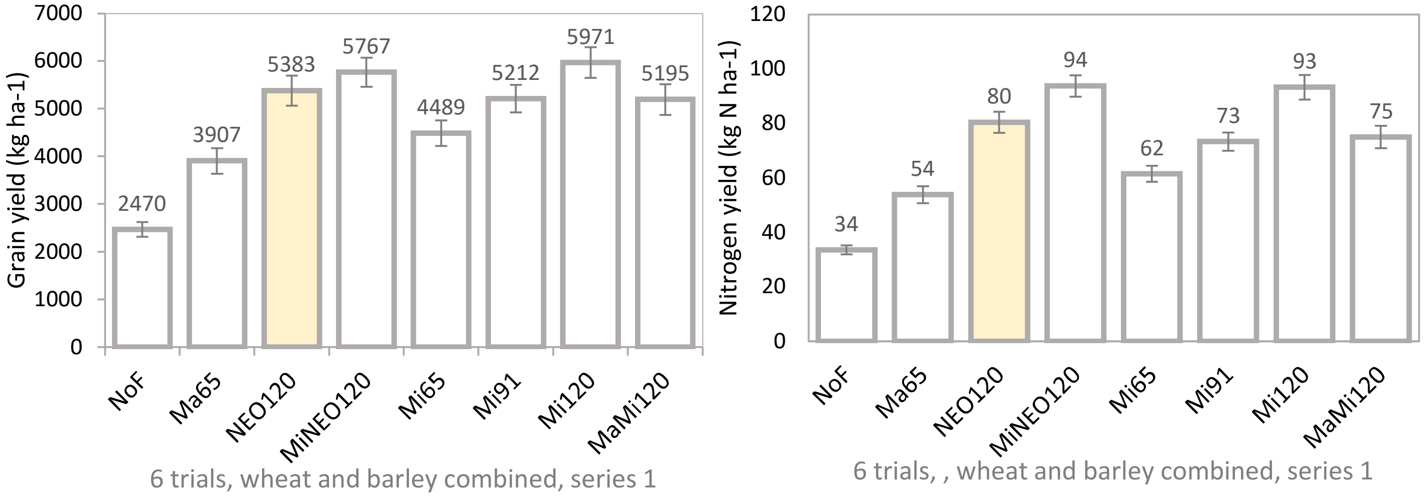


Figure 20: Grain yield (15 % water content, kg ha^{-1}) (left), and Nitrogen yield (kg N ha^{-1}) (Right) ± standard error of the means—results from Series 1 in 2021 and 2022, six trials, wheat and barley combined.

Furthermore, the observed pattern paralleled grain yield among treatments regarding barley N yield. However, considering wheat N yield, MiNEO120 exhibited the highest N yield at 106.1 kg N ha^{-1}. This outcome significantly surpassed both NEO120 and MaMi120. In addition, it had an insignificant 8.7 kg N ha-1 advantage over Mi120 (Figure 20).

3.4.2. Barley and wheat grain and nitrogen yield – series 2

In the context of Series Two, the barley grain yield of MaMi120 was 5064 kg ha^{-1}, akin to the yield of Mi120. However, Mi120 exhibited a slightly higher but insignificant yield of 170 kg ha^{-1} over NEO120; NEO120 yielded between the extents of Mi105 and Mi120, with a significantly higher yield than Ma65 (Figure 21).

Regarding the wheat grain yield, MaMi120 significantly outperformed NEO120 by 451 kg ha^{-1}. In contrast, NEO120's yield aligned with that of Mi105, but it demonstrated a significant superiority compared to Ma65 (Figure 21). Thus, a similar pattern was observed for barley and wheat N yield. However, the variations between treatments were more pronounced, reflecting an increased divergence in N yield (Figure 21).

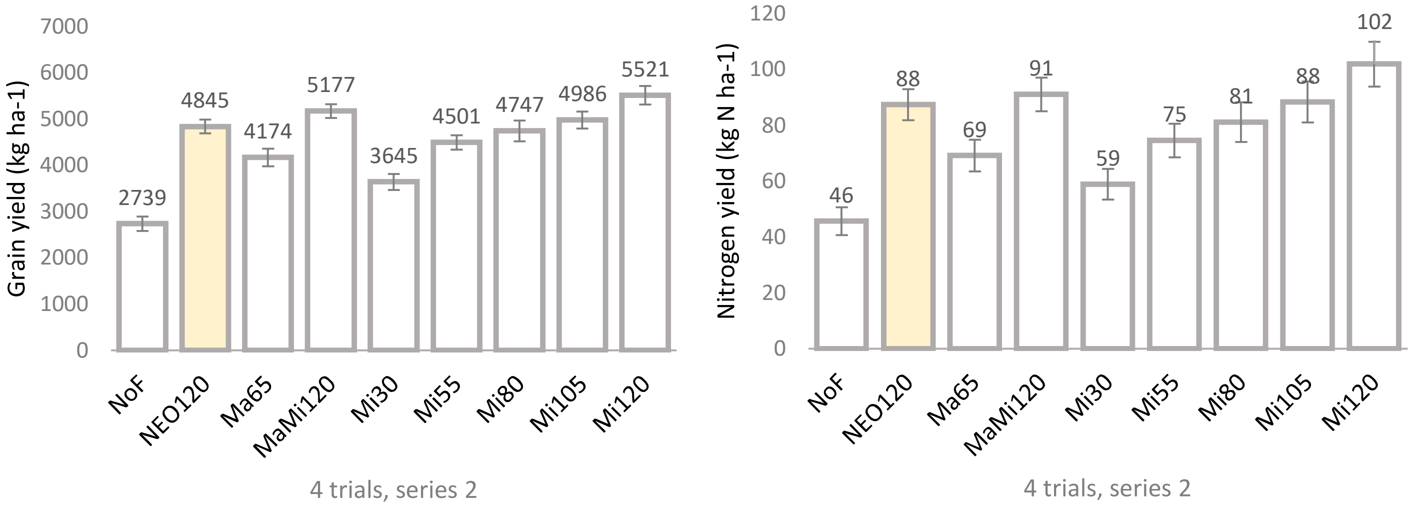


Figure 21: Grain yield (15 % water content, kg ha^{-1}) (left), and Nitrogen yield (kg N ha^{-1}) (Right) ± standard error of the means—results from Series 2 in 2021 and 2022, four trials, wheat and barley combined.

3.4.3. Sum up all average yields

When consolidating outcomes across all barley and wheat trials, MaMi120 exhibited a barley grain yield of 5083 kg ha^{-1} and a wheat grain yield of 5290 kg ha^{-1}. These results equated to the barley yield of NEO120, while MaMi120 outperformed NEO120 by 135 kg ha^{-1} for wheat. MaMi120 and NEO120 yielded over 1000 kg ha^{-1} more than Ma65. Mi120 achieved a barley grain yield 425 kg ha^{-1} higher and a wheat grain yield 968 kg ha^{-1} higher than NEO120.

Focusing exclusively on Series 1, akin yield discrepancies appeared as in the cumulative results. In barley, MiNEO120 aligned with NEO120, whereas in wheat, it outperformed NEO120 by 743 kg ha^{-1} and MaMi120 by 935 kg ha^{-1}. Interestingly, MiNEO120 even surpassed Mi120 in wheat grain yield.

In short, NEO120 demonstrated a similar impact on grain yield as 95 kg N ha^{-1} in mineral fertilizer and an equivalent effect on N yield as 100 kg N ha^{-1} in mineral fertilizer. To simplify, when considering wheat and barley grain yield, replacing 95 kg N ha^{-1} in mineral fertilizer with 120 kg N ha^{-1} in NEO is realistic. Likewise, 100 kg N-min ha^{-1} in mineral fertilizer can be exchanged for 120 kg N-min ha^{-1} in NEO for N yield assessment.

Ma65 displayed a grain yield effect akin to 50 kg N ha^{-1} in mineral fertilizer, although the N yield from Ma65 slightly trailed. MaMi120 also displayed the same grain yield N effect as 95 kg N ha^{-1} in mineral fertilizer. However, the N yield from MaMi120 was marginally lower in comparison.

4. Discussion

The production of Nitrogen-Enriched Organic fertilizers (NEO) is an innovative technology supplementing atmospheric N in biobased fertilizers. The technology could potentially hold promise for sustainable agriculture by reducing agriculture's carbon footprint by using N from the air and converting it into a form that plants can easily absorb.

However, to fully achieve the benefits of this technology, it is essential to prioritize using renewable energy sources, which can significantly reduce our dependence on fossil fuel-based fertilizers, a significant contributor to greenhouse gas emissions (Li et al., 2018; Rouwenhorst et al., 2021; Tsonev et al., 2023). Nonetheless, before NEO can be scaled and commercialized, it is crucial to thoroughly evaluate its potential impact on soil life and crop yields.

This requires comprehensive explorations under both field and laboratory conditions to map and document any adverse effects that may arise. Furthermore, it is essential to explore this new fertilizer's competitiveness in crop productivity compared to other alternatives farmers have.

By identifying and addressing potential risks associated with using NEO, we can ensure it is safe, effective, and environmentally friendly. This highlights the significance of responsible innovation within the agricultural sector, underscoring the need for thorough evaluation and refinement of innovative technologies to mitigate any potential adverse effects on soil health and ecosystem services.

The impact of N fertilization on soil faunal communities, their population dynamics, and feeding activity is well researched. However, whether soil-dwelling organisms can adapt to external factors remains elusive (Birkhofer, Baulechner, Diekötter, Zaitsev, & Wolters, 2022), particularly in the case of a newly developed fertilizer such as NEO. Even though NEO is biobased, it holds substantial nitrite content and low pH, which can hypothetically form harmful radicals and toxic compounds. Thus, assessing NEO's potential adverse impact on soil biota by different approaches is crucial.

Thus, our studies aimed to investigate and compare the effects of fertilization with NEO to commonly used mineral and organic fertilizers on soil fauna feeding activity, the abundance of springtails, the abundance and weight of earthworms, soil nitrification potential, and crop yields. This was to highlight the effects of NEO on soil functions and biota and to document its safety and agronomic efficacy before widespread adoption in agriculture.

4.1. NEO and soil fauna feeding activity

Our results revealed that low doses of fertilizer positively affected feeding activity in the early weeks after fertilizer application, regardless of the type of fertilizer used. However, high doses of fertilizer had a slightly negative effect on feeding activity. These findings are consistent with a grassland study that showed decreased feeding activity in response to excessive amounts of organic fertilizer (Birkhofer et al., 2022).

In the mid-term evaluation 14 weeks after fertilization, the pattern was similar to the early effect evaluation but with more minor differences across fertilization treatments. Additionally, high concentrations of NEO showed a slightly increased soil faunal feeding activity compared to no fertilizer. However, the initial stimulation gradually started to fade over time after fertilization.

During the late effect evaluation 21 weeks after fertilization, the initial positive effect of low fertilizer levels on feeding activity disappeared. The difference among treatments was much smaller than in the short- and mid-term evaluations, with less than a five percent difference between the highest and lowest feeding activities.

Although higher amounts of fertilizer initially showed a negative effect on soil faunal feeding activity, this detrimental effect gradually stabilized with time after fertilization. This stabilizing effect has also been reported in a previous study (Wahyuningsih et al., 2019), indicating the soil's buffering capacity and other soil chemical responses that diminish fertilization's perturbative effect. Moreover, soil fauna may be functionally redundant, conveying resilience to transient perturbations. In summary, neither NEO nor conventional fertilizers used in the experiment adversely affected soil faunal feeding activity.

In contrast, the results from our other trials were inconsistent with those mentioned above, where we observed that excessive amounts of fertilizers indicated high soil fauna feeding activity. Despite the findings, it is essential to note that the studies were focused on determining whether NEO harms the soil fauna's functional activity compared to other fertilization treatments.

Therefore, it is rational to conclude that using NEO did not negatively impact soil fauna-feeding activity compared to other fertilizers and that factors beyond fertilization may control soil fauna-feeding activity. Hence, it is critical to explore further the complex interactions between soil fauna, fertilization, and other environmental factors.

4.2. NEO and key species in the soil

4.2.1. Springtails

During the summer, a few weeks after fertilization, the abundance of springtails was almost equal in the cereal field plots fertilized with organic fertilizer and NEO. The abundance was slightly lower in the plots fertilized with mineral fertilizer. However, in the grass field, the plots fertilized with organic fertilizer exhibited nearly double and quadrupled the number of springtails compared to those fertilized with NEO and mineral fertilizer. Similarly, during the fall in the cereal and grass fields, there were slightly more springtails in the plots fertilized with organic fertilizer and no fertilizer compared to the plots fertilized with NEO and mineral fertilizer; however, the differences were not notable.

Our study did not find a decrease in the abundance of springtails due to organic fertilization, which contradicts a previous study that indicated a reduction in springtail abundance following cattle slurry application (Pommeresche et al., 2017). Interestingly, another study also found almost no effect of fertilization on the abundance of springtails (Gergócs et al., 2022), while a different study reported an increase in the number of springtails following fertilization (S. Wang, Chen, Tan, Fan, & Ruan, 2016). After all, our study found no harmful impact of NEO or other fertilizers on the abundance of springtails.

Additionally, the abundance of springtails was generally lower during summer than in fall. There are two potential explanations for this inconsistency. Given that springtails are moisture-dependent organisms (Machado, Oliveira Filho, Santos, Paulino, & Baretta, 2019), sampling on a warm, dry day with limited moisture in the surface soil may have forced a significant portion of the springtail community to move deeper into the soil to avoid desiccation (Butterfield, 1999). Similarly, more decaying plant matter may be available more profoundly in the soil after harvest, which could serve as a source of food for springtails (Christiansen, Bellinger, & Janssens, 2009).

4.2.2. Earthworms

The effects of agricultural amendments on soil biota can be investigated in the short and long run. In our studies, we utilized our developed method to investigate and compare the immediate effects (8 days) of fertilization with NEO and other fertilizers on earthworms' abundance and average total weight.

Results from both experiments revealed that none of the fertilizer treatments harmed earthworm abundance or weight after eight days. The control group that received no fertilizer was the only treatment with a slight reduction in abundance and weight. Interestingly, comparable results were obtained in both years, except for the fact that the no-fertilizer treatment did not result in any reduction in earthworm abundance or weight during the second year.

We expected that adding organic matter to the soil through NEO and organic fertilizer would promote the earthworm population (Clive A Edwards & Bohlen, 1996; Pommeresche & Løes, 2009). On the other hand, there was a concern that excessive liquid slurry in a single dose might harm the earthworms (C. A. Edwards & Lofty, 1982). Nonetheless, this did not occur in our experiments.

In addition, mineral fertilizers may directly or indirectly benefit earthworms (C. A. Edwards & Lofty, 1982; Hendrix et al., 1992; Tiwari, 1993). Conversely, ammonium-based fertilizers can also potentially lower soil pH and adversely affect earthworms in the long run (D. Ernst, 1995), but none of these were observed in our experiments.

The slight increase in the number of earthworms observed in our experimental plots after eight days might raise concerns regarding the accuracy of our findings. However, we must note that we implemented measures to prevent earthworms from escaping, ensuring consistency across all experimental plots. Consequently, we can assert that any potential sources of error were identical for all plots. Therefore, based on these considerations, it is reasonable to conclude that applying fertilization with NEO or any other fertilizer did not have a restrictive effect on the earthworm population in the soil.

4.3. NEO and nitrification potential in agricultural soils

Nitrification is a vital process that converts ammonium into nitrite and nitrate, an essential nutrient for plant growth. We evaluated changes in potential nitrification by monitoring soil microbial activity after fertilization with NEO and other fertilizers (Hu, Shen, Xu, & Zheng, 2011; Robertson, 2015).

4.3.1. Field fertilized soil

The nitrification rate evaluation of field-fertilized soil samples collected in autumn, 5-6 months after fertilization, revealed no significant differences between fertilization treatments in the cereal and grass fields.

Perhaps the extended period between fertilization and sample collection allowed for removing added N from the soil through plant uptake or other measures, restricting potential effects. Therefore, we can conclude that the application of NEO does not have a long-lasting impact on soil nitrification, even after two consecutive years of application.

Based on previous studies, we expected to observe a distinction in potential nitrification rates between fertilized and unfertilized soil (Mohanty et al., 2022; Raglin, Soman, Ma, & Kent, 2022). Surprisingly, this distinction was not observed in our experiments. This could be due to the relatively short two-year treatment period or nitrification-sustaining mechanisms, such as N mining from the relatively high soil organic matter (SOM) content in the unfertilized treatment. Further treatment years would be necessary to draw definitive conclusions regarding the long-term effects of fertilization, including NEO.

In addition, we observed lower nitrification rates in the grass field soil than in the cereal field soil. This was expected due to the pH-dependent nature of nitrification (DeForest & Otuya, 2020), and the grassland soil had a significantly lower pH than the cereal field.

4.3.2. Lab fertilized soil

The utilization of NEO derived from biogas digestate (NEO D) and cattle slurry (NEO S) yielded a pronounced enhancement in the stimulation of nitrification rates. This effect surpassed the impact of alternative fertilization treatments in cereal and grass field soils. These soils were supplemented with fertilizer and incubated as agitated soil slurries. The pattern was also observed in loosely placed lab-fertilized soil samples, although the nitrification rates were lower than the agitated soil slurries. Once again, NEO D led to more stimulation of nitrification rates than other fertilization treatments. However, unlike in the agitated soil slurries, the nitrification rates with NEO S and ammonium chloride were almost identical in the loosely placed lab-fertilized soil samples. This confirms that active nitrifier communities are not solely dependent on ammonium accessibility, as hypothesized in the study conducted by Raglin et al. (2022).

One would expect lower nitrification rates in NEO slurries due to product inhibition caused by considerably higher NO_2^- and NO_3^- contents and lower pH values than untreated slurries.

Interestingly, the opposite was found, indicating that NEO stimulates nitrification transiently regardless of its NH_4^+ content through some other mechanism.

Although the organo-chemical composition of NEO is unknown, it is arguable that the plasma process forms radicals that create volatile organic compounds (VOCs), which were found to stimulate nitrification in one study (Mohanty et al., 2019). The fact that the same stimulation pattern was observed across treatments in two different soils suggests that NEO has a factor related to it, independent of soil.

Another potential reason for the stimulation by NEO could be that its C/N ratio is smaller than that of untreated cattle slurry or biogas digestate due to N enrichment. A smaller C/N ratio reduces the immobilization of NH_4^+ and sustains a higher nitrification rate (Watson, Atkinson, Gosling, Jackson, & Rayns, 2002).

Although the NEO's acidic nature was presumed to slow down nitrification temporarily, nitrification rates increased with the addition of NH_4^+ in our lab experiments. That being said, this effect was more pronounced with NEO D and NEO S than in other amendments. It is imperative to emphasize that the assessments of nitrification were conducted under carefully controlled laboratory conditions, which may differ from the complexities of real-world dynamics.

In contrast, nitrification potentials in NEO field treatments were indistinguishable from those of other fertilizer treatments after two years of application, suggesting that NEO's low pH or high nitrite content does not harm soil nitrifiers' activity throughout the year. Therefore, our studies suggest NEO could be potentially harmless as a bio-based fertilizer in agroecosystems. Nonetheless, we only investigated the effects of NEO and other fertilizers on nitrification rates in two sub-boreal soils.

Thus, to broaden our understanding of the physiochemical drivers and changes in soil microbial communities, future studies should explore the taxonomic composition of nitrifier communities under fertilization. This will enable us to map the mechanisms regulating nitrification rates under different fertilization treatments. It will eventually provide valuable insights into the complex interactions between soil microorganisms and their environment, leading to a more nuanced understanding of soil ecology and nutrient cycling under fertilization.

4.4. NEO and crop yields

We have also thoroughly explored the impacts of NEO on Italian ryegrass yields under controlled conditions and its effects on cereals under field conditions. Our study was designed to comprehensively address and compare the influence of NEO on crop yields in both controlled and field environments. A series of pot and field experiments were meticulously conducted to accomplish this objective, examining NEO alongside various levels and combinations of mineral and organic fertilizers, particularly cattle slurry.

The primary observation emerging from our controlled conditions study was the anticipated positive correlation between N application and ryegrass yields. As mineral fertilization levels increased, so did the yield, reaffirming existing knowledge of the beneficial impact of mineral N on plant growth (Abraha, Truter, Annandale, & Fessehazion, 2015; Cinar, Özkurt, & Cetin, 2020; Harris, Thom, & Clark, 1996).

Notably, the designed N ladder in mineral fertilizer application served as a benchmark to evaluate the fertilization efficacy of NEO. When applied at 175 kg N-min ha^{-1}, NEO demonstrated yields within the same range or slightly lower than mineral fertilizers with identical N content. Therefore, this outcome underscores NEO's potential as a viable alternative.

Comparative analyses between NEO and untreated manure unveiled NEO's advantage, with 31–36% higher yields achieved through NEO application. Notably, despite variations in the nitrite and nitrate percentages within different NEO types, all NEO variants produced similar yields. This suggests that total N content is more decisive in determining yield outcomes.

Our findings align with previous studies documenting the positive relationship between mineral N and ryegrass yields (Cinar et al., 2020). Not surprisingly, the highest yields were attained at maximum fertilization levels, with results congruent with studies conducted on different soil types (Abraha et al., 2015). These results were consistent even on clay soils in different geographical contexts. While specific studies have reported superior effects when combining mineral and organic fertilizers (Körschens et al., 2013), our observations did not replicate this outcome. Albeit acknowledging that experiments conducted under controlled conditions possess inherent limitations when set against field studies is crucial.

Compatible with our findings under controlled conditions, our field study focusing on cereals and assessing NEO's capacity as an N alternative to mineral fertilizers revealed that the application of 120 kg N-min ha^{-1} in NEO yielded grain outcomes like those obtained with 95 kg N-min ha^{-1} in mineral fertilizer. This outcome indicates a reduction of 20% in yield compared to the use of 120 kg N-min ha^{-1} in mineral fertilizer. Furthermore, when considering N yield, applying 120 kg N-min ha^{-1} in NEO demonstrated a performance that closely resembled the outcomes achieved with 100 kg N-min ha^{-1} in mineral fertilizer, resulting in a reduction of approximately 16.7% (Amon et al., 2022; Jackson & Smith, 1997; Ladha, Pathak, J. Krupnik, Six, & van Kessel, 2005).

Furthermore, applying 65 kg N-min ha^{-1} in untreated manure alongside 55 kg N-min ha^{-1} in mineral fertilizer yielded results equivalent to using 95 kg N-min ha^{-1} in mineral fertilizer and 120 kg N-min ha^{-1} in NEO for both barley and wheat. Notably, within 120 kg N-min ha^{-1} in NEO, 60 kg N-min was introduced to the manure through plasma treatment. This suggests that NEO has the potential to replace the combination of untreated manure and mineral fertilizers, offering a strategy to decrease reliance on mineral fertilizers while sustaining comparable yields.

Although even higher yields were achieved when solely employing mineral fertilizers, it is crucial to highlight that NEO consistently yielded 20–30% more than the native amount of cattle slurry from which it originated. This implies an advantage in enhancing crop productivity, achieved through only electricity and cattle slurry as inputs.

However, the unexpected outcomes observed regarding crop yield discrepancies between NEO and mineral fertilizers call for further exploration. While the low pH in NEO is commonly presumed to mitigate ammonia loss (Fangueiro, Hjorth, & Gioelli, 2015), it might not be the sole contributing factor. The elevated soil nitrification potential shortly after NEO application observed in our other study (Mousavi et al., 2023) could arguably result in ineffective plant uptake. Undeniably, it is necessary to restate that the evaluations of nitrification were executed within the laboratory's optimized conditions, which may deviate from the dynamics of real-world scenarios.

Nevertheless, it remains noteworthy that approximately 15-25% of the N contained within NEO does not translate directly to crop yields. Consequently, undertaking a comprehensive research initiative becomes imperative to elucidate this phenomenon, pinpoint where the irrecoverable N is directed, and understand the potential environmental ramifications of NEO.

5. Conclusion and future perspectives

Our studies investigated and compared the effects of newly developed Nitrogen-Enriched Organic fertilizer (NEO) to other conventional agricultural amendments. This was to determine the effects of NEO on crop yields and that it does not harm biota in agricultural soils.

In brief, the results indicated that NEO fertilizers did not negatively affect soil fauna feeding activity, springtails' abundance, or earthworms' abundance and weight. Moreover, although NEO immediately stimulated soil nitrification in the laboratory, the effect was not sustained under field conditions.

Furthermore, regarding crop productivity, an equivalent N-min amount in NEO resulted in slightly less grass yields than mineral fertilizers under controlled conditions. Consistent with those results, NEO exhibited a cereal yield approximately 20% lower than mineral fertilizer in the field. However, it is worth highlighting that NEO demonstrated a remarkable advantage, yielding 20–30% more than the native cattle slurry from which it was derived.

Thus, utilizing NEO as a biobased fertilizer could yield advantages such as initiating soil nitrification during the initial stages of the growth cycle and amplifying plant growth. Nonetheless, it is vital to consider the potential downside of increased nitrate availability.

Further, our studies suggest additional long-term exploration to comprehensively understand the impact of NEO on soil nitrification and other soil quality indicators. Besides, our results could be supplemented by investigating the impact of NEO on other soil-dwelling organisms, e.g., nematodes and mites, and the overall soil ecosystem's health. Eventually, molecular methods, e.g., metabarcoding, could be employed to map and compare the impact of NEO on soil-living communities to other conventional fertilizers.

After all, it is vital to acknowledge that NEO is an innovative product undergoing continuous refinements and quality enhancements, primarily focusing on bolstering sustainability and resilience within agricultural ecosystems while mitigating the product's overall climate impact. The insights from our research play a pivotal role in shaping the development of a sustainable and environmentally friendly NEO, thereby contributing significantly to the ongoing refinement process.

6. Acknowledgment

At the pinnacle of my academic journey, I stand here humbled and overwhelmed with gratitude as I pen down these words of acknowledgment. This moment would not have been possible without the unwavering support, love, and guidance of countless individuals who have left an indelible mark on my life and this thesis.

First and foremost, I extend my most profound appreciation to my supervisors, Prof. Svein Øivind Solberg and Assistant Prof. Thomas Cottis, whose unwavering belief in my potential has been a constant source of inspiration. Also, my heartfelt gratitude to Prof. Peter Dörsch for his significant contributions to my research. Your invaluable guidance, patience, and endless encouragement have shaped me into the researcher I am today. Your unwavering commitment to excellence and tireless efforts in helping me navigate the intricate labyrinth of academia has been nothing but extraordinary.

To my family, whose unconditional love, sacrifices, and unwavering belief in me have been my guiding light. Your constant encouragement and unwavering faith in my abilities have given me the strength to persevere, even in adversity.

To my colleagues and friends, both near and far, who have accompanied me on this arduous journey, thank you for your camaraderie, laughter, and unwavering support. I extend my heartfelt gratitude to the faculty and staff at Inland University of Applied Sciences (INN), whose tireless dedication to fostering an environment conducive to learning and growth has shaped me into the scholar I am today.

To my dissertation evaluation committee, Prof. Charles A. Francis, Research Scientist Bente Føreid, and Associate Prof. Peder Lombnæs, I am indebted to your expertise and invaluable insights. Your constructive criticism and thoughtful suggestions have elevated the quality of this work. I am grateful for the intellectual challenges you presented, which have pushed me to broaden my horizons and strive for excellence in every aspect of my research.

This dissertation stands as a testament to the collective efforts of all those who have touched my life profoundly. Your belief in me, unwavering support, and unconditional love propelled me forward when the path seemed daunting.

With a heart overflowing with gratitude,
Hesam Mousavi
October 27th, 2023, Hamar, Norway

7. References

Abraha, A. B., Truter, W. F., Annandale, J. G., & Fessehazion, M. K. (2015). Forage yield and quality response of annual ryegrass (Lolium multiflorum) to different water and nitrogen levels. *African Journal of Range & Forage Science, 32*(2), 125-131. doi:10.2989/10220119.2015.1056228

Amon, B., Stoumann, L., Groenestein, K., Döhler, H., Sobrino, O., Sutton, M., . . . Cordovil, C. (2022). UNECE Guidance Document on Integrated Sustainable Nitrogen Management Nitrogen Opportunities for Agriculture, Food & Environment. In (pp. 57).

Augustin, M. A., Riley, M., Stockmann, R., Bennett, L., Kahl, A., Lockett, T., . . . Cobiac, L. (2016). Role of food processing in food and nutrition security. *Trends in Food Science & Technology, 56*, 115-125. doi:https://doi.org/10.1016/j.tifs.2016.08.005

Basso, B., Dumont, B., Cammarano, D., Pezzuolo, A., Marinello, F., & Sartori, L. (2016). Environmental and economic benefits of variable rate nitrogen fertilization in a nitrate vulnerable zone. *Science of The Total Environment, 545-546*, 227-235. doi:10.1016/j.scitotenv.2015.12.104

Bell, C. W., Asao, S., Calderon, F., Wolk, B., & Wallenstein, M. D. (2015). Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biology and Biochemistry, 85*, 170-182. doi:10.1016/j.soilbio.2015.03.006

Berlese, A. (1905). Apparecchio per raccogliere presto ed in gran numero piccoli arthropodi [Apparatus for collecting early and large numbers of small arthropods]. In *Redia* (Vol. 2): Harvard University. (Reprinted from: 16 Jun 2008).

Birkhofer, K., Baulechner, D., Diekötter, T., Zaitsev, A., & Wolters, V. (2022). Fertilization Rapidly Alters the Feeding Activity of Grassland Soil Mesofauna Independent of Management History. *Frontiers in Ecology and Evolution, 10*. doi:10.3389/fevo.2022.864470

Bondada, B. R., & Oosterhuis, D. M. (2001). Canopy photosynthesis, specific leaf weight, and yield components of cotton under varying nitrogen supply. *Journal of Plant Nutrition, 24*(3), 469-477. doi:10.1081/PLN-100104973

Bottinelli, N., Hedde, M., Jouquet, P., & Capowiez, Y. (2020). An explicit definition of earthworm ecological categories – Marcel Bouché's triangle revisited. *Geoderma, 372*, 114361. doi:10.1016/j.geoderma.2020.114361

Bouché, M. B. (1977). Strategies lombriciennes. *Ecological Bulletins*(25), 122-132.

Brightling, J. (2018). Ammonia and the fertiliser industry: The development of ammonia at Billingham. *Johnson Matthey Technology Review, 62*(1), 32-47. doi:10.1595/205651318X696341

Bünemann, E. K., Schwenke, G. D., & Van Zwieten, L. (2006). Impact of agricultural inputs on soil organisms—a review. *Soil Research, 44*(4). doi:10.1071/sr05125

Butterfield, J. (1999). Changes in decomposition rates and Collembola densities during the forestry cycle in conifer plantations. *Journal of Applied Ecology, 36*(1), 92-100. doi:10.1046/j.1365-2664.1999.00382.x

Chen, K.-E., Chen, H.-Y., Tseng, C.-S., & Tsay, Y.-F. (2020). Improving nitrogen use efficiency by manipulating nitrate remobilization in plants. *Nature Plants, 6*(9), 1126-1135. doi:10.1038/s41477-020-00758-0

Christiansen, K. A., Bellinger, P., & Janssens, F. (2009). Chapter 55 - Collembola: (Springtails, Snow Fleas). In V. H. Resh & R. T. Cardé (Eds.), *Encyclopedia of Insects (Second Edition)* (pp. 206-210). San Diego: Academic Press.

Cinar, S., Özkurt, M., & Cetin, R. (2020). Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (Lolium multiflorum l.) in central black sea climatic zone in turkey. *Applied Ecology and Environmental Research, 18*, 417-432. doi:10.15666/aeer/1801_417432

Coleman, D. C., & Wall, D. H. (2015). Chapter 5 - Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function. In E. A. Paul (Ed.), *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)* (pp. 111-149). Boston: Academic Press.

Cottis, T., Mousavi, H., & Solberg, S. Ø. (2023). Plasma Treated Cattle Slurry Moderately Increases Cereal Yields. *Agronomy, 13*(6), 1549. doi:doi:10.3390/agronomy13061549

Crawford, N. M., & Forde, B. G. (2002). Molecular and developmental biology of inorganic nitrogen nutrition. *Arabidopsis Book, 1*, e0011. doi:10.1199/tab.0011

Cui, Z., Zhang, H., Chen, X., Zhang, C., Ma, W., Huang, C., . . . Dou, Z. (2018). Pursuing sustainable productivity with millions of smallholder farmers. *Nature, 555*(7696), 363-366. doi:10.1038/nature25785

DeForest, J. L., & Otuya, R. K. (2020). Soil nitrification increases with elevated phosphorus or soil pH in an acidic mixed mesophytic deciduous forest. *Soil Biology and Biochemistry, 142*, 107716. doi:10.1016/j.soilbio.2020.107716

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication. *Nature, 418*(6898), 700-707. doi:10.1038/nature01019

Diaz, C., Saliba-Colombani, V., Loudet, O., Belluomo, P., Moreau, L., Daniel-Vedele, F., . . . Masclaux-Daubresse, C. (2006). Leaf Yellowing and Anthocyanin Accumulation are Two Genetically Independent Strategies in Response to Nitrogen Limitation in Arabidopsis thaliana. *Plant and Cell Physiology, 47*(1), 74-83. doi:10.1093/pcp/pci225

Ding, L., Wang, K. J., Jiang, G. M., Biswas, D. K., Xu, H., Li, L. F., & Li, Y. H. (2005). Effects of Nitrogen Deficiency on Photosynthetic Traits of Maize Hybrids Released in Different Years. *Annals of Botany, 96*(5), 925-930. doi:10.1093/aob/mci244

Doane, T. A., & Horwáth, W. R. (2011). Spectrophotometric Determination of Nitrate with a Single Reagent. *Analytical Letters, 36*(12), 2713-2722. doi:10.1081/AL-120024647

Dong, N.-Q., & Lin, H.-X. (2020). Higher yield with less nitrogen fertilizer. *Nature Plants, 6*(9), 1078-1079. doi:10.1038/s41477-020-00763-3

Döös, B. R. (2002). Population growth and loss of arable land. *Global Environmental Change, 12*(4), 303-311. doi:10.1016/S0959-3780(02)00043-2

Edwards, C. A. (1977). *Biology of earthworms / C. A. Edwards, J. R. Lofty*. London : New York: Chapman and Hall ; Wiley.

Edwards, C. A., & Bohlen, P. J. (1996). *Biology and ecology of earthworms* (Vol. 3): Springer Science & Business Media.

Edwards, C. A., & Lofty, J. R. (1982). Nitrogenous fertilizers and earthworm populations in agricultural soils. *Soil Biology and Biochemistry, 14*(5), 515-521. doi:10.1016/0038-0717(82)90112-2

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience, 1*(10), 636-639. doi:10.1038/ngeo325

Ernst, D. (1995). *The Farmer's Earthworm Handbook: Managing Your Underground Money-Makers*. Brookfield, Wi: Lessiter Publications, Inc.

Ernst, F. A. (1928). *Fixation of atmospheric nitrogen*. Journal of the Society of Chemical Industry: Chapman & Hall, Ltd.

Eulene Francisco da, S., Marlenildo Ferreira, M., Kássio Ewerton Santos, S., Tatiane Severo, S., Diana Ferreira de, F., Maria Eugênia da, C., . . . Paula Romyne de Morais

Cavalcante, N. (2019). Organic Nitrogen in Agricultural Systems. In R. Everlon Cid & S. Ademar Pereira (Eds.), *Nitrogen Fixation* (pp. Ch. 2). Rijeka: IntechOpen.

European Commission. (2022). Sustainable use of nutrients: The common agricultural policy supports farmers in the safe and efficient use of nutrients. *Nutrients in EU agriculture.* Retrieved from https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability/low-input-farming/nutrients_en

European Commission, Environment, D.-G. f., Toland, J., Sliva, J., Camarsa, G., Rosskopf, N., . . . Nottingham, S. (2014). *LIFE and soil protection*: Publications Office.

Eyde, H. S. (1909). The manufacture of nitrates from the atmosphere by the electric arc—Birkeland-Eyde process. *Journal of the Royal Society of Arts, 57*(2949), 568-576.

Fangueiro, D., Hjorth, M., & Gioelli, F. (2015). Acidification of animal slurry– a review. *Journal of Environmental Management, 149*, 46-56. doi:https://doi.org/10.1016/j.jenvman.2014.10.001

FAO. (2019). *World fertilizer trends and outlook to 2022*. Rome, Italy: FAO.

FAO. (2022a, 27.02.2022). Fertilizers indicators. *FAOSTAT.* 2021. Retrieved from https://www.fao.org/faostat/en/#data/EF

FAO. (2022b). *The State of Knowledge of Soil Biodiversity: Enter a hidden world*. Retrieved from https://www.fao.org/resources/digital-reports/soil-biodiversity/en/: https://www.fao.org/resources/digital-reports/soil-biodiversity/en/

Frink, C. R., Waggoner, P. E., & Ausubel, J. H. (1999). Nitrogen fertilizer: Retrospect and prospect. *Proceedings of the National Academy of Sciences, 96*(4), 1175-1180. doi:10.1073/pnas.96.4.1175

Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., . . . Menta, C. (2009). Soil biodiversity monitoring in Europe: ongoing activities and challenges. *European Journal of Soil Science, 60*(5), 807-819. doi:10.1111/j.1365-2389.2009.01177.x

Geisseler, D., Lazicki, P. A., & Scow, K. M. (2016). Mineral nitrogen input decreases microbial biomass in soils under grasslands but not annual crops. *Applied Soil Ecology, 106*, 1-10. doi:10.1016/j.apsoil.2016.04.015

Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biology and Biochemistry, 75*, 54-63. doi:https://doi.org/10.1016/j.soilbio.2014.03.023

Gergócs, V., Flórián, N., Tóth, Z., Szili-Kovács, T., Mucsi, M., & Dombos, M. (2022). Crop species and year affect soil-dwelling Collembola and Acari more strongly than fertilisation regime in an arable field. *Applied Soil Ecology, 173*, 104390. doi:10.1016/j.apsoil.2022.104390

Ghaly, A., & vasudevan ramakrishnan, V. (2015). Nitrogen sources and cycling in the ecosystem and its role in air, water and soil pollution: A critical review. *Journal of Pollution Effects & Control, 3*.

Good, A. G., Shrawat, A. K., & Muench, D. G. (2004). Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science, 9*(12), 597-605. doi:10.1016/j.tplants.2004.10.008

Graenitz, J., & Bauer, R. (2000). The effect of fertilization and crop rotation on biological activity in a 90 year long-term experiment. *Bodenkultur, 51*, 99-105.

Graff, O. (1984). Unsere Regenwürmer (Lexikon für Freunde der Bodenbiologie) [Our earthworms (lexicon for friends of soil biology)]. *Journal of Plant Nutrition and Soil Science, 147*(4), 530-530. doi:10.1002/jpln.19841470411

Graves, D. B., Bakken, L. B., Jensen, M. B., & Ingels, R. (2019). Plasma Activated Organic Fertilizer. *Plasma Chemistry and Plasma Processing, 39*(1), 1-19. doi:10.1007/s11090-018-9944-9

Griess, P. (1858). Vorläufige Notiz über die Einwirkung von salpetriger Säure auf Amidinitro- und Aminitrophenylsäure [Preliminary note on the action of nitrous acid on amidinitro- and aminitrophenylic acid]. *Annalen der Chemie und Pharmacie [Annals of Chemistry and Pharmacy], 106*, 123-125.

Guðleifsson, B. (2002). Impact of long term use of fertilizer on surface invertebrates in experimental plots in a permanent hayfield in Northern-Iceland. *Agricultural Society of Iceland, 15*.

Hamel, C., Schellenberg, M. P., Hanson, K., & Wang, H. (2007). Evaluation of the "bait-lamina test" to assess soil microfauna feeding activity in mixed grassland. *Applied Soil Ecology, 36*(2-3), 199-204. doi:10.1016/j.apsoil.2007.02.004

Harris, S. L., Thom, E. R., & Clark, D. A. (1996). Effect of high rates of nitrogen fertiliser on perennial ryegrass growth and morphology in grazed dairy pasture in northern New Zealand. *New Zealand Journal of Agricultural Research, 39*(1), 159-169. doi:10.1080/00288233.1996.9513174

Hättenschwiler, S., Tiunov, A. V., & Scheu, S. (2005). Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics, 36*(1), 191-218. doi:10.1146/annurev.ecolsys.36.112904.151932

Hedberg, T. (2020). *The environmental impact of overpopulation: the ethics of procreation* (1 ed.): Routledge.

Hendrix, P. F., Mueller, B. R., Bruce, R. R., Langdale, G. W., & Parmelee, R. W. (1992). Abundance and distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A. *Soil Biology and Biochemistry, 24*(12), 1357-1361. doi:10.1016/0038-0717(92)90118-H

Herren, G. L., Habraken, J., Waeyenberge, L., Haegeman, A., Viaene, N., Cougnon, M., . . . Bert, W. (2020). Effects of synthetic fertilizer and farm compost on soil nematode community in long-term crop rotation plots: A morphological and metabarcoding approach. *PLoS One, 15*(3), e0230153. doi:10.1371/journal.pone.0230153

Hopkin, S. P. (1997). *Biology of the springtails (Insecta--Collembola)*: Oxford University Press, 1997.

Houlton, B. Z., Almaraz, M., Aneja, V., Austin, A. T., Bai, E., Cassman, K. G., . . . Zhang, X. (2019). A World of Cobenefits: Solving the Global Nitrogen Challenge. *Earth's Future, 7*(8), 865-872. doi:doi.org/10.1029/2019EF001222

Hu, B. L., Shen, L. D., Xu, X. Y., & Zheng, P. (2011). Anaerobic ammonium oxidation (anammox) in different natural ecosystems. *Biochemical Society Transactions, 39*(6), 1811-1816. doi:10.1042/bst20110711

Ingels, R., & Graves, D. B. (2015). Improving the Efficiency of Organic Fertilizer and Nitrogen Use via Air Plasma and Distributed Renewable Energy. *Plasma Medicine, 5*(2-4), 257-270. doi:10.1615/PlasmaMed.2016015763

Isleib, J. (2017). A quick look at the nitrogen cycle and nitrogen fertilizer sources – Part 2. *Various nitrogen fertilizers fit different needs.* Retrieved from https://www.canr.msu.edu/news/nitrogen_cycle_and_nitrogen_fertilizer_sources_part_2

ISO18311(E). (2015). Soil quality – Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms in the field – Bait-lamina test" for approval for the next stage (FDIS) until 2015-04-17 (see Recommendation Paris-2).

Jackson, D. R., & Smith, K. A. (1997). Animal manure slurries as a source of nitrogen for cereals; effect of application time on efficiency. *Soil Use and Management, 13*(2), 75-81. doi:https://doi.org/10.1111/j.1475-2743.1997.tb00560.x

Janker, J., Mann, S., & Rist, S. (2019). Social sustainability in agriculture – A system-based framework. *Journal of Rural Studies, 65*, 32-42. doi:https://doi.org/10.1016/j.jrurstud.2018.12.010

Jänsch, S., Scheffczyk, A., & Römbke, J. (2017). The bait-lamina earthworm test: a possible addition to the chronic earthworm toxicity test? *Euro-Mediterranean Journal for Environmental Integration, 2*(1). doi:10.1007/s41207-017-0015-z

Keeney, D. R., & Nelson, D. W. (1983). Nitrogen—Inorganic Forms. In A. L. Page (Ed.), *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* (pp. 643-698): American Society of Agronomy, Inc., Soil Science Society of America, Inc.

Kellogg Biological. (2019). Gravimetric Soil Moisture. *Protocols, Long-term ecological research.* Retrieved from https://lter.kbs.msu.edu/protocols/24

Kiiski, H., Scherer, H. W., Mengel, K., Kluge, G., & Severin, K. (2009). Fertilizers, 1. General. In *Ullmann's Encyclopedia of Industrial Chemistry* (pp. 1-30): Wiley Online Library.

Killham, K. (1998). Soil Microbiology and Biochemistry. By E. A. PAUL and F. E. CLARK. In *New Phytologist* (Vol. 138, pp. 563-566). San Diego, CA, USA: Academic Press: 2nd Edition,.

Körschens, M., Albert, E., Armbruster, M., Barkusky, D., Baumecker, M., Behle-Schalk, L., . . . Zorn, W. (2013). Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: results from 20 European long-term field experiments of the twenty-first century. *Archives of Agronomy and Soil Science, 59*(8), 1017-1040. doi:10.1080/03650340.2012.704548

Kratz, W. (1998). Bait-lamina test, general aspect, applications and perspectives. *Environmental Science and Pollution Research, 5*(2), 3. doi:10.1007/BF02986394

Ladha, J. K., Pathak, H., J. Krupnik, T., Six, J., & van Kessel, C. (2005). Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects. In *Advances in Agronomy* (Vol. 87, pp. 85-156): Academic Press.

Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., & Garnier, J. (2014). 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters, 9*(10), 105011. doi:10.1088/1748-9326/9/10/105011

Li, S., Medrano, J. A., Hessel, V., & Gallucci, F. (2018). Recent Progress of Plasma-Assisted Nitrogen Fixation Research: A Review. *Processes, 6*(12), 248. doi:10.3390/pr6120248

Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data, 9*(1), 181-192. doi:10.5194/essd-9-181-2017

Machado, J., Oliveira Filho, L. C., Santos, J., Paulino, A., & Baretta, D. (2019). Morphological diversity of springtails (Hexapoda: Collembola) as soil quality bioindicators in land use systems. *Biota Neotropica, 19*, e20180618. doi:10.1590/1676-0611-bn-2018-0618

Mann, S. (1983, 1983//). *Mineralization in biological systems.* Paper presented at the Inorganic Elements in Biochemistry, Berlin, Heidelberg.

Marschner, H. (2002a). 4 - Uptake and Release of Mineral Elements by Leaves and Other Aerial Plant Parts. In H. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Second Edition)* (pp. 116-130). San Diego: Academic Press.

Marschner, H. (2002b). 6 - Mineral Nutrition and Yield Response. In H. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Second Edition)* (pp. 184-200). San Diego: Academic Press.

Marschner, H. (2002c). 13 - Nutrient Availability in Soils. In H. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Second Edition)* (pp. 483-507). San Diego: Academic Press.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Ann Bot, 105*(7), 1141-1157. doi:10.1093/aob/mcq028

Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science, 277*(5325), 504-509. doi:10.1126/science.277.5325.504

Mc Laughlin, A., & Mineau, P. (1995). The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment, 55*(3), 201-212. doi:https://doi.org/10.1016/0167-8809(95)00609-V

McAllister, C. H., Beatty, P. H., & Good, A. G. (2012). Engineering nitrogen use efficient crop plants: the current status. *Plant biotechnology journal, 10*(9), 1011-1025. doi:10.1111/j.1467-7652.2012.00700.x

Meier, F. A., Scherrer, S., & Honegger, R. (2002). Faecal pellets of lichenivorous mites contain viable cells of the lichen-forming ascomycete Xanthoria parietina and its green algal photobiont, Trebouxia arboricola. *Biological Journal of the Linnean Society, 76*(2), 259-268. doi:10.1046/j.1095-8312.2002.00065.x

Miller, A. J., & Cramer, M. D. (2005). Root Nitrogen Acquisition and Assimilation. *Plant and Soil, 274*(1), 1-36. doi:10.1007/s11104-004-0965-1

Mohanty, S. R., Nagarjuna, M., Parmar, R., Ahirwar, U., Patra, A., Dubey, G., & Kollah, B. (2019). Nitrification Rates Are Affected by Biogenic Nitrate and Volatile Organic Compounds in Agricultural Soils. *Frontiers in Microbiology, 10*. doi:10.3389/fmicb.2019.00772

Mohanty, S. R., Parihar, M., Dubey, G., Parmar, R., Jain, D., Singh, M., . . . Kollah, B. (2022). Nitrification under the influence of long-term fertilizer application in a tropical vertisol. *Archives of Agronomy and Soil Science, 68*(4), 516-530. doi:10.1080/03650340.2020.1843022

Montanarella, L., Pennock, D. J., McKenzie, N., Badraoui, M., Chude, V., Baptista, I., . . . Vargas, R. (2016). World's soils are under threat. *SOIL, 2*(1), 79-82. doi:10.5194/soil-2-79-2016

Mousavi, H., Cottis, T., Hoff, G., & Solberg, S. Ø. (2022). Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. *Sustainability, 14*(4), 2005. doi:doi:10.3390/su14042005

Mousavi, H., Cottis, T., Pommeresche, R., Dörsch, P., & Solberg, S. Ø. (2022). Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. *Agronomy, 12*(10), 2314. doi:doi:10.3390/agronomy12102314

Mousavi, H., Solberg, S. Ø., Cottis, T., & Dörsch, P. (2023). Nitrogen-Enriched Organic fertilizer (NEO) elevates nitrification rates shortly after application but has no lasting effect on nitrification in agricultural soils. doi:10.21203/rs.3.rs-2565156/v2

Mueller, N. D., Gerber, J. S., Johnston, M., Ray, D. K., Ramankutty, N., & Foley, J. A. (2012). Closing yield gaps through nutrient and water management. *Nature, 490*(7419), 254-257. doi:10.1038/nature11420

Mulvaney, R., Khan, S., & Ellsworth, T. (2009). Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. *Journal of environmental quality, 38*(6), 2295-2314. doi:10.2134/jeq2008.0527

Mulvaney, R. L. (1996). Nitrogen—Inorganic Forms. In *Methods of Soil Analysis* (pp. 1123-1184).

Muratore, C., Espen, L., & Prinsi, B. (2021). Nitrogen Uptake in Plants: The Plasma Membrane Root Transport Systems from a Physiological and Proteomic Perspective. *Plants, 10*(4), 681.

N2 Applied. (2022). Nitrogen Enriched Organic fertiliser. Retrieved from https://n2applied.com/

National Geographic Society. (2022, 2022.07.08). The Development of Agriculture. Retrieved from https://education.nationalgeographic.org/resource/development-agriculture/12th-grade/

O'Connor, F. B. (1962). The extraction of Enchytraeidae from soil. In *Progress in Soil Zoology* (pp. 279-285). Butterworth, London.,: EurekaMag.

Öhlinger, R., Eibelhuber, A., & Vinzenz, M. (1993). Die Verteilung und Streuung von Bodenenzymaktivitäten in einer einheitlichen Fläche und deren Einfluß auf Probenahme und Analyse [The distribution and scatter of soil enzyme activities in a uniform area and their influence on sampling and analysis]. *Die Bodenkultur - Journal for Land Management, Food and Environment, 44*(2).

Pelosi, C., Boros, G., van Oort, F., & Schmidt, O. (2020). Soil Oligochaeta communities after 9 decades of continuous fertilization in a bare fallow experiment. *SOIL ORGANISMS, 92*(2), 129–141. doi:10.25674/so92iss2pp129

Pimentel, D. (2006). Soil Erosion: A Food and Environmental Threat. *Environment, Development and Sustainability, 8*(1), 119-137. doi:10.1007/s10668-005-1262-8

Pommeresche, R., & Løes, A.-K. (2009). Relations between agronomic practice and earthworms in Norwegian arable soils. *Global Science Books*.

Pommeresche, R., Løes, A.-K., & Torp, T. (2017). Effects of animal manure application on springtails (Collembola) in perennial ley. *Applied Soil Ecology, 110*, 137-145. doi:10.1016/j.apsoil.2016.10.004

Prăvălie, R., Patriche, C., Borrelli, P., Panagos, P., Roșca, B., Dumitrașcu, M., . . . Bandoc, G. (2021). Arable lands under the pressure of multiple land degradation processes. A global perspective. *Environmental Research, 194*, 110697. doi:10.1016/j.envres.2020.110697

Raglin, S. S., Soman, C., Ma, Y., & Kent, A. D. (2022). Long Term Influence of Fertility and Rotation on Soil Nitrification Potential and Nitrifier Communities. *Frontiers in Soil Science, 2*. doi:10.3389/fsoil.2022.838497

Ritchie, H. (2021). Can we reduce fertilizer use without sacrificing food production? *Reducing fertilizer use.* Retrieved from https://ourworldindata.org/reducing-fertilizer-use

Ritchie, H., Roser, M., & Rosado, P. (2022, 26.4.2023). Fertilizers. Retrieved from https://ourworldindata.org/fertilizers

Robertson, G. P., Groffmanand P. M. (2015). 14 - NITROGEN TRANSFORMATIONS. In E. A. Paul (Ed.), *Soil Microbiology, Ecology and Biochemistry,* (Fourth edition ed., pp. 421-426): Academic Press, Burlington, Massachusetts, USA.

Roser, M., Ritchie, H., Ortiz-Ospina, E., & Rodés-Guirao, L. (2013). World Population Growth. Retrieved from https://ourworldindata.org/world-population-growth

Roser M., & Ritchie, H. (2019, 18.06.2019). Two centuries of rapid global population growth will come to an end. Retrieved from https://ourworldindata.org/world-population-growth-past-future

Rouwenhorst, K. H. R., Jardali, F., Bogaerts, A., & Lefferts, L. (2021). From the Birkeland-Eyde process towards energy-efficient plasma-based NO (X) synthesis: a techno-economic analysis. *Energy Environ Sci, 14*(5), 2520-2534. doi:10.1039/d0ee03763j

Rożen, A., Sobczyk, Ł., Liszka, K., & Weiner, J. (2010). Soil faunal activity as measured by the bait-lamina test in monocultures of 14 tree species in the Siemianice common-garden experiment, Poland. *Applied Soil Ecology, 45*(3), 160-167. doi:10.1016/j.apsoil.2010.03.008

Russel, D. A., & Williams, G. G. (1977). History of Chemical Fertilizer Development. *Soil Science Society of America Journal, 41*(2), 260-265. doi:10.2136/sssaj1977.03615995004100020020x

Rütting, T., Aronsson, H., & Delin, S. (2018). Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems, 110*(1), 1-5. doi:10.1007/s10705-017-9900-8

Schinner, F., Öhlinger, R., Kandeler, E., & Margesin, R. (1996). *Methods in Soil Biology* (1 ed.). Berlin: Springer-Verlag.

Siebert, J., Sunnemann, M., Auge, H., Berger, S., Cesarz, S., Ciobanu, M., . . . Eisenhauer, N. (2019). The effects of drought and nutrient addition on soil organisms vary across taxonomic groups, but are constant across seasons. *Sci Rep, 9*(1), 639. doi:10.1038/s41598-018-36777-3

Silva, D. M. d., Jacques, R. J. S., Silva, D. A. A. d., Santana, N. A., Vogelmann, E., Eckhardt, D. P., & Antoniolli, Z. I. (2016). Effects of pig slurry application on the diversity and activity of soil biota in pasture areas. *Ciência Rural, 46*, 1756-1763.

Simpson, J. E., Slade, E., Riutta, T., & Taylor, M. E. (2012). Factors Affecting Soil Fauna Feeding Activity in a Fragmented Lowland Temperate Deciduous Woodland. *PLoS One, 7*(1), e29616. doi:10.1371/journal.pone.0029616

Sims, R. W., Gerard, B. M., London, L. S., & Association, E. B. W. S. (1985). *Earthworms: Keys and Notes for the Identification and Study of the Species*: Linnean Society of London and the Estuarine and Brackish-Water Sciences Association.

Smil, V. (2004). *Enriching the earth: Fritz Haber, Carl Bosch, and the transformation of world food production*: MIT press.

Stitt, M., & Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment, 22*(6), 583-621. doi:10.1046/j.1365-3040.1999.00386.x

Sundermeier, A. (2016). Manure and Compost: Nitrogen Availability in Organic Production. *Agriculture and Natural Resources.* Retrieved from https://ohioline.osu.edu/factsheet/anr-34

Tao, H.-H., Slade, E. M., Willis, K. J., Caliman, J.-P., & Snaddon, J. L. (2016). Effects of soil management practices on soil fauna feeding activity in an Indonesian oil palm plantation. *Agriculture, Ecosystems & Environment, 218*, 133-140. doi:10.1016/j.agee.2015.11.012

Terra Protecta. (2020, 2020). The Bait-Lamina Test. Retrieved from http://www.terra-protecta.de/en/bait_strips.html

Thion, C., & Prosser, J. I. (2014). Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying–rewetting stress. *FEMS Microbiology Ecology, 90*(2), 380-389. doi:10.1111/1574-6941.12395

Tiwari, S. C. (1993). Effects of organic manure and NPK fertilization on earthworm activity in an Oxisol. *Biology and Fertility of Soils, 16*(4), 293-295. doi:10.1007/BF00369307

Törne, E. v. (1990). Assessing feeding activities of soil-living animals. Bait-lamina-tests. *Pedobiologia, 34*(2), 89-101.

Travis, A. S., Travis, A. S., & Costa. (2018). *Nitrogen capture*: Springer.

Tsonev, I., O'Modhrain, C., Bogaerts, A., & Gorbanev, Y. (2023). Nitrogen Fixation by an Arc Plasma at Elevated Pressure to Increase the Energy Efficiency and Production Rate of NOx. *ACS Sustainable Chemistry & Engineering, 11*(5), 1888-1897. doi:10.1021/acssuschemeng.2c06357

Tullgren. (1918). Ein sehr einfacher Ausleseapparat für terricole Tierformen [A very simple elite apparatus for terricole animal forms]. *Zeitschrift für Angewandte Entomologie [Journal of Applied Entomology], 4*(1), 149-150. doi:10.1111/j.1439-0418.1918.tb00820.x

United Nations. (2017). The World Population Prospects: The 2017 Revision. *Department of Economic and Social Affairs.* Retrieved from https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100#:~:text=COVID%2D19-,World%20population%20projected%20to%20reach%209.8%20billion%20in%202050%2C%20and,Nations%20report%20being%20launched%20today.

United Nations. (2019). Meetings Coverage and Press Releases. Retrieved from https://press.un.org/en/2019/sgsm19680.doc.htm#:~:text=Every%20year%2C%20the%20world%20loses,weakened%20resilience%20to%20climate%20change.

United Nations. (2020a). Contribution of life in soil 'remains largely underestimated'. *Climate and Environment.* Retrieved from https://news.un.org/en/story/2020/12/1079352#:~:text=Even%20though%20soil%20organisms%20play,(FAO)%20said%20on%20Friday.

United Nations. (2020b). Fertilizers: challenges and solutions. Retrieved from https://www.unep.org/news-and-stories/story/fertilizers-challenges-and-solutions

United Nations. (2022). Population. *GLOBAL ISSUES.* Retrieved from https://www.un.org/en/global-issues/population

Upendra, M. S., Rajan, G., & Gautam, P. P. (2019). Nitrogen Fertilization I: Impact on Crop, Soil, and Environment. In R. Everlon Cid & S. Ademar Pereira (Eds.), *Nitrogen Fixation* (pp. Ch. 5). Rijeka: IntechOpen.

Verhoef, H. A., & Brussaard, L. (1990). Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. *Biogeochemistry, 11*(3), 175. doi:10.1007/BF00004496

Vermeir, I., & Verbeke, W. (2006). Sustainable Food Consumption: Exploring the Consumer "Attitude – Behavioral Intention" Gap. *Journal of Agricultural and Environmental Ethics, 19*(2), 169-194. doi:10.1007/s10806-005-5485-3

Wahyuningsih, R., Marchand, L., Pujianto, S., & Caliman, J. (2019). Impact of inorganic fertilizer to soil biological activity in an oil palm plantation. *IOP Conference Series: Earth and Environmental Science, 336*, 012017. doi:10.1088/1755-1315/336/1/012017

Wang, S., Chen, H. Y. H., Tan, Y., Fan, H., & Ruan, H. (2016). Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. *Scientific Reports, 6*(1), 20816. doi:10.1038/srep20816

Wang, Z. (2010). Griess Diazotization, Z. Wang (Ed.). In *Comprehensive Organic Name Reactions and Reagents* (pp. 1267-1270).

Watson, C. A., Atkinson, D., Gosling, P., Jackson, L. R., & Rayns, F. W. (2002). Managing soil fertility in organic farming systems. *Soil Use and Management, 18*(s1), 239-247. doi:10.1111/j.1475-2743.2002.tb00265.x

Wei, M., Hu, G., Wang, H., Bai, E., Lou, Y., Zhang, A., & Zhuge, Y. (2017). 35 years of manure and chemical fertilizer application alters soil microbial community composition in a Fluvo-aquic soil in Northern China. *European Journal of Soil Biology, 82*, 27-34. doi:10.1016/j.ejsobi.2017.08.002

Winter, L. R., & Chen, J. G. (2021). N2 Fixation by Plasma-Activated Processes. *Joule, 5*(2), 300-315. doi:10.1016/j.joule.2020.11.009

Yara. (2021a). YaraLiva™. Retrieved from https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaraliva/

Yara. (2021b). YaraMila™. Retrieved from https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaramila/

yr.no. (2023). Weather data history. from Norwegian Meteorological Institute and the Norwegian Broadcasting Corporation www.yr.no

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research, 14*(6), 415-421. doi:https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Dissertation articles

1. Mousavi, H., Cottis, T., Hoff, G., & Solberg, S. Ø. (2022). Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. Sustainability, 14(4), 2005. doi: 10.3390/su14042005

2. Mousavi, H., Cottis, T., Pommeresche, R., Dörsch, P., & Solberg, S. Ø. (2022). Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. Agronomy, 12(10), 2314. doi: 10.3390/agronomy12102314

3. Mousavi, H., Solberg, S. Ø., Cottis, T., & Dörsch, P. (2023). Nitrogen-Enriched Organic fertilizer (NEO) elevates nitrification rates shortly after application but has no lasting effect on nitrification in agricultural soils. doi:10.21203/rs.3.rs-2565156/v2.

4. Cottis, T., Mousavi, H., & Solberg, S. Ø. (2023). Plasma Treated Cattle Slurry Moderately Increases Cereal Yields. Agronomy, 13(6), 1549. doi: 10.3390/agronomy13061549

sustainability
IMPACT FACTOR 3.9
CITESCORE 5.8

1

Article

Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions

Hesam Mousavi, Thomas Cottis, Gina Hoff and Svein Øivind Solberg

Special Issue
Advances in Sustainable Agricultural Crop Production
Edited by
Prof. James W. Muthomi, Dr. Alex M. Fulano and Dr. Nancy Karimi Njeru

MDPI

https://doi.org/10.3390/su14042005

sustainability

MDPI

Article

Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions

Hesam Mousavi [1,2,*], Thomas Cottis [1], Gina Hoff [1] and Svein Øivind Solberg [1]

[1] Department of Agricultural Sciences, Faculty of Applied Ecology and Biotechnology, Inland Norway University of Applied Sciences, 2318 Hamar, Norway; thomas.cottis@inn.no (T.C.); ginamhoff@gmail.com (G.H.); svein.solberg@inn.no (S.Ø.S.)

[2] Gothenburg Global Biodiversity Center (GGBC), The University of Gothenburg, SE-405 30 Gothenburg, Sweden

* Correspondence: hemousavi65@gmail.com

Abstract: This study aimed to investigate the effects of a new nitrogen-enriched organic-based fertilizer (NEO) on Italian ryegrass (*Lolium multiflorum* Lam.) yield and soil fauna feeding activity. Nitrogen is transformed from the air to manure by a plasma process. At the farm level, NEO could improve self-sufficiency and sustainability. The work was carried out under controlled conditions in two pot trials. Five fertilization regimes were used: no fertilizer, different amounts of mineral fertilizer, three NEO types, organic fertilizer (untreated manure), and organic fertilizer + different amounts of N in mineral fertilizer, including 14 treatments in trial one and 11 treatments in trial two. Besides evaluating dry matter yields, we utilized the Bait-lamina test system to assess the feeding activity of soil fauna. The results indicated a clear positive impact of nitrogen (N) on ryegrass yield where all fertilizers increased the yield in correspondence with their N availability regardless of the fertilizer type; whereas the yield was highest with mineral fertilizer up to our maximum level of 235 kg N ha^{-1} in trial one and 175 kg N ha^{-1} in trial two. The NEO fertilizers yielded in the same range as mineral fertilizers. The same clear pattern was not observed for soil fauna feeding activity. Instead, a tendency was observed where no fertilization tends to give the highest feeding activity. We saw no correlation between the yield and the soil fauna feeding activity. The feeding activity was highest in depth below 5 cm from the soil surface. Feeding activity also increased over time after fertilization. The NEO fertilizers had no more adverse effects on soil fauna feeding activity than other fertilizers. Other factors than fertilization alone are determining the soil fauna feeding activity.

Keywords: fertilization; nutrients; nitrogen; NEO; ryegrass; soil health; sustainability; yield

Citation: Mousavi, H.; Cottis, T.; Hoff, G.; Solberg, S.Ø. Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. *Sustainability* **2022**, *14*, 2005. https://doi.org/10.3390/su14042005

Academic Editors: James W. Muthomi, Alex M. Fulano and Nancy Karimi Njeru

Received: 14 January 2022
Accepted: 7 February 2022
Published: 10 February 2022

1. Introduction

Agriculture systems are extensively nourished with mineral fertilizers [1], and the global input of nitrogen (N) into the biological system is eight times higher today than it was in the 1960s [1–3]. Although fertilization has contributed to higher yields, the trade-offs are many and severe [3,4]. Therefore, sustainable use of fertilizers is of significant interest, and combining mineral and organic fertilizers or improving the efficiency of applied fertilizers are ways to maintain good yields and sustain soils [5]. The positive effect of N fertilizers on yield is documented well [6–8], but its effects on soil organisms and functions are more complex and often neglected [9,10]. Fertilization directly manipulates soil nutrients and stimulates alteration in soil functional communities, making the environment favorable for some functional groups and more unfavorable for others [11]. These functional groups are engaged in plants' well-being and ecosystem services in different ways, such as disease protection, pathogenicity, and nutrient turn-over [12]. Thus, identifying a fertilizer regime with the most negligible negative impact on soil functional organisms is of high priority and potentially leads to more sustainable agriculture.

Grasses are considered an important option for cover and forage crops in the Nordic conditions [13]. Perennial and annual ryegrasses (*Lolium* spp.) are among the most robust grasses in the region and are commonly used in agriculture fields and grasslands [14,15]. Nevertheless, having a fast growth rate, extended roots, high yields, high forage quality, and low tillage requirement, make annual ryegrasses a favorable crop in forage production and as rotation crops [16].

Nitrogen Enriched Organic fertilizer (NEO) is a newly patented organic-based fertilizer. Using electricity with a patented unit, nitrogen is transformed from the air to the manure by a plasma process. The manufacturer (N2 Applied, Asker, Norway) claims that the final product can be a suitable alternative for conventional organic and mineral fertilizers [17–19]. NEO enables end-users to produce their fertilizer locally and provides self-sufficiency, improving plant production sustainability [17]. NEO holds the same characteristics as cattle slurry but contains more nitrite and nitrate, has lower pH, and is more fluid due to filtration in the production process. As a result, NEO is claimed to have considerably lower emissions than other liquid fertilizers and can be dozed more precisely [18]. The current study is part of a fine-tuning work with N2 Applied, where we examine different NEO types and compare their effects with mineral fertilizers and untreated slurry. A particular focus is on the effects of fertilizers on soil biological activity.

Theoretically, fertilizers improve soil biological activity through increased crop production and crop residue return [10]. On the other hand, N fertilizers, particularly ammonium-N, may increase soil acidy, leading to reduced biological activity or shifts in the functional groups in the soil [10,20,21]. Moreover, repeated applications could theoretically suppress certain soil enzymes involved in nutrient cycles, e.g., the amidase involved in the N cycle [20]. Studies show diminished soil microbial biomass under high N application rates in grassland, presumably through reduced plant species richness. A similar pattern has not been noticed under annual crop cultivations [9,22,23]. Nevertheless, the way soil functional groups confront recurrent mineral fertilization depends on different environmental and management factors [21].

Organic amendments, e.g., manure, compost, biosolids, and humic substances, serve as the carbon source for soil organisms, directly by their carbon content and indirectly via improved plant growth and plant residue returns. Overall, recurrent applications of organic fertilizers have promoted soil microbial growth and activity [24] and enhanced plant productivity [9]. The impacts of organic fertilizers on the microbial communities are more favorable than those of chemical fertilizers and vary between annual and perennial production systems [24]. Regarding soil fauna and invertebrates, their vulnerability to high N application rates differs. Detrimental effects on soil fauna feeding activity have been reported in both the short term [9,20] and long term [25]. However, other studies showed positive effects of fertilization on soil fauna composition and diversity and the feeding activity of the organisms [22,23,25]. These positive effects were evident in springtails in the topsoil [26]. Nevertheless, these controversies demonstrate the importance of more research to understand the changes in soil biota after fertilizers.

The current study investigates the effects of different fertilizers, including mineral fertilizer, NEO, and combinations of organic and mineral fertilizers, on ryegrass yields and soil fauna feeding activity under controlled conditions. The aims are: (1) to identify a fertilizer regime with optimal yields and compare NEOs' effect on yields to mineral fertilizer and organic fertilizer; and (2) to investigate the impact of the fertilizers on the soil fauna feeding activity. Here, NEO, as a new fertilizer, is our primary target.

2. Materials and Methods

2.1. Experiment Design

Pot experiments were conducted in a growing chamber at Inland Norway University of Applied Sciences. We used perforated pots (13 × 13 × 18 cm) filled with 2.5 L of field soil and fertilized them simultaneously. Each fertilizing treatment was randomized within four replicates.

Two subsequent trials were performed. Trial one consisted of 14 fertilizing treatments, and trial two was somewhat simplified with 11 to investigate and compare the effects of lower and more adjacent amounts of mineral fertilizer to the NEO (Table 1). The treatments were five fertilizing regimes; no fertilizer; different amounts of mineral fertilizer (Yara Mila 18-3-15) [27]; three types of NEO (N2 Applied) [17]; organic fertilizer (untreated cattle slurry); and organic fertilizer + different amounts of N in mineral fertilizer (Yara Liva 16-0-0) [28]. The mineral fertilizer levels were designed as a ladder from no fertilizer to a maximum of 235 kg N ha^{-1} in trial one and 175 kg N ha^{-1} in trial two. The Yara mineral fertilizers were added in the solid form; however, untreated slurry and all NEO types were added in liquid form. Treatments in trial one were (1) No fertilizer, (2) Mineral fertilizer 115 kg N ha^{-1}, (3) Mineral fertilizer 145 kg N ha^{-1}, (4) Mineral fertilizer 175 kg N ha^{-1}, (5) Mineral fertilizer 205 kg N ha^{-1}, (6) Mineral fertilizer 235 kg N ha^{-1}, (7) NEO type A 175.4 kg N ha^{-1}, (8) NEO type B 175.4 kg N ha^{-1}, (9) NEO type C 175.4 kg N ha^{-1}, (10) Organic fertilizer 73 kg N ha^{-1}, (11) Organic fertilizer + MF 115 kg N ha^{-1}, (12) Organic fertilizer + MF 145 kg N ha^{-1}, (13) Organic fertilizer + MF 175 kg N ha^{-1}, and (14) Organic fertilizer + MF 205 kg N ha^{-1}. Treatments in trial two were (1) No fertilizer, (2) Mineral fertilizer 60 kg N ha^{-1}, (3) Mineral fertilizer 80 kg N ha^{-1}, (4) Mineral fertilizer 115 kg N ha^{-1}, (5) Mineral fertilizer 135 kg N ha^{-1}, (6) Mineral fertilizer 155 kg N ha^{-1}, (7) Mineral fertilizer 175 kg N ha^{-1} (8) NEO type A 175.4 kg N ha^{-1}, (9) NEO type B 175.4 kg N ha^{-1}, (10) NEO type C 175.4 kg N ha^{-1}, and (11) Organic fertilizer 73 kg N ha^{-1}.

Furthermore, the three types of NEO were different in terms of acidity, nitrate, and nitrite contents. The pH of NEO type A was 5.42 and contained 1530 mg L^{-1} NH_4^+, 800 mg/L NO_2^- and 1180 mg L^{-1} NO_3^-. NEO type B had pH 5.35 and contained 1480 L^{-1} NH_4^+, 777 L^{-1} NO_2^-, and 1250 L^{-1} NO_3^-. NEO type C had pH 4.24 and contained 1100 L^{-1} NH_4^+, 444 L^{-1} NO_2^- and 1910 L^{-1} NO_3^-. Moreover, the cattle slurry used in this experiment had pH 7.13, and it contained 1320 L^{-1} NH_4^+. The target untreated manure amount was 55 tons ha^{-1}. The N2 Applied unit filters all particles larger than 5 mm during the production process, which reduces the total amount by 10 percent, down to 50 tons ha^{-1}. The untreated manure had 1.33 kg plant-available N per ton. However, after processing by the N2-Applied unit, the final product (NEO) had a plant-available N content of 3.51 kg per ton.

We used soil taken from the experimental farm. According to the soil analysis report from Eurofins (https://www.eurofins.no/agro-testing/ (accessed on 30 January 2022)) on samples taken prior to the experiment, the soil texture was sandy clay loam with over 10% clay and 4.5% soil organic matter. The soil pH was high (pH = 7.4) while the phosphorus status was normal (P-AL = 11 mg/100 g), and the potassium status was low (K-AL = 5 mg/100 g). The field water capacity was estimated to be 33.6% of soil volume and with a pore capacity of 41.4%.

Pot preparation and planting were done following the procedure that we developed. First, a moistened paper tissue was placed at the bottom of the perforated pots to avoid soil eruption through the holes. Then, 0.6 L (5 cm) soil was filled into the pots. The second layer, 0.8 L (6 cm) soil, was fertilized and filled into the pots. Fertilizer amounts were based on the recommended field application rates (in tons per hectare), calculated for 169 cm^2 soil surface per pot (Table 1). Next, an additional 0.9 L (6 cm) soil was filled in the pots, and seeds were placed over the top. Thirty-six Italian ryegrass seeds (*Lolium multiflorum* Lam.), variety 'Barpluto' (NAK Nederland/Ref. DE148-214011) were seeded with 12 seeds in three rows per pot. Then additional 0.2 L (1 cm) soil was filled on the top to form the outermost soil layer.

We used a Lumatek ATS300W LED lighting system (https://lumatek-lighting.com/ (accessed on 30 January 2022)). The LED light pads provided a full spectrum of light (380–780 nm wavelength) suitable for growing cereals and grasses under controlled conditions [29]. For this experiment, five LED pads were positioned in a row and 35 cm over the top foliage. The LED pads were lifted as the plants grew. According to a typical Nordic summer day length, the light/dark duration was set to 16 h light and 8 h dark. Moreover, light intensity was measured using a digital light meter for approving equal light access for 16 pots per lamp (80 × 80 cm). The temperature in the growing chamber was 16 °C during this experiment.

Table 1. Fertilizing treatments used in trials one and two with the different fertilization treatments in different colors and detailed application rates.

Trial One	**Fertilizing Treatment**	**Organic Fertilizer (tons ha^{-1})**	**Kg N in Yara Mila18-3-15 (kg ha^{-1})**	**Kg N in Organic Fertilizer (kg ha^{-1})**	**Kg N in Yara Liva 16-0-0 (kg ha^{-1})**	**Total kg N (kg ha^{-1})**
1	No fertilizer	-	-	-	-	0
2	Mineral fertilizer 115 kg N ha^{-1}		115			115
3	Mineral fertilizer 145 kg N ha^{-1}		145			145
4	Mineral fertilizer 175 kg N ha^{-1}		175			175
5	Mineral fertilizer 205 kg N ha^{-1}		205			205
6	Mineral fertilizer 235 kg N ha^{-1}		235			235
7	NEO type A 175.4 kg N ha^{-1}	50		175.4		175.4
8	NEO type B 175.4 kg N ha^{-1}	50		175.4		175.4
9	NEO type C 175.4 kg N ha^{-1}	50		175.4		175.4
10	Organic fertilizer 73 kg N ha^{-1}	55		73		73
11	Organic fertilizer + MF 115 kg N ha^{-1}	55		73	42	115
12	Organic fertilizer + MF 145 kg N ha^{-1}	55		73	72	145
13	Organic fertilizer + MF 175 kg N ha^{-1}	55		73	102	175
14	Organic fertilizer + MF 205 kg N ha^{-1}	55		73	132	205
Trial Two	**Fertilizing Treatment**	**Organic Fertilizer (tons ha^{-1})**	**Kg N in Yara Mila18-3-15 (kg ha^{-1})**	**Kg N in Organic Fertilizer (kg ha^{-1})**	**Kg N in Yara Liva 16-0-0 (kg ha^{-1})**	**Total kg N (kg ha^{-1})**
1	No fertilizer	-	-	-	-	0
2	Mineral fertilizer 60 kg N ha^{-1}		60			60
3	Mineral fertilizer 80 kg N ha^{-1}		80			80
4	Mineral fertilizer 115 kg N ha^{-1}		115			115
5	Mineral fertilizer 135 kg N ha^{-1}		135			135
6	Mineral fertilizer 155 kg N ha^{-1}		155			155
7	Mineral fertilizer 175 kg N ha^{-1}		175			175
8	NEO type A 175.4 kg N ha^{-1}	50		175.4		175.4
9	NEO type B 175.4 kg N ha^{-1}	50		175.4		175.4
10	NEO type C 175.4 kg N ha^{-1}	50		175.4		175.4
11	Organic fertilizer 73 kg N ha^{-1}	55		73		73

2.2. *Growing Conditions and Yield*

Primary irrigation was done with 500 mL water to provide adequate moisture. This amount was 55% of field capacity for our dry soil, whereas the soil was not wholly dry at planting. During plants' growth, the irrigation routine was 200 mL water three times a week for the first weeks. Irrigation intervals were 200 mL every 1–2 days during the last weeks before harvest progressing the plant developmental stages [30].

The pots were placed adhering to each other for the first two weeks. A five cm distance was applied between pots from week three to prevent plants from competing for light and space. After germination, the least vigor plants in each row were thinned, allowing 24 plants per pot (eight plants per row). A few weeds germinated per pot during the experiment, and these were removed by hand.

In trial one, plants were harvested six weeks after planting. Moreover, a second harvest took place three weeks after the first harvest. Harvesting was done by cutting the plants 1 cm from the soil surface. Bulk yield and dry matter yield (DM) from each pot were measured at harvest and after drying at 60 °C for 48 h [31]. In trial two, a single harvest was done eight weeks after planting. Otherwise, the same procedure was followed as described for trial one.

2.3. Feeding Activity of Soil Fauna

The soil fauna feeding activity was assessed using Bait-lamina strips (Terra Protecta GmbH, Berlin, Germany) [32]. The Bait-lamina test is an efficient, prompt, and replicable method with high statistical relevance via several replications [33,34]. This method evaluates soil fauna feeding activity as a decisive function in nutrient cycling [34–36]. The method has revealed promising results regarding the soil fauna feeding activity for screening and comparing them under different managements and practices [34]. The method exposes perforated PVC strips (1 mm × 6 mm × 120 mm) with 16 holes of 1.5 mm diameter with 5 mm distance filled with a bait substrate to soil fauna (invertebrates) feeding activity. The substrate consists of 70% cellulose powder, 25% wheat bran, and 5% activated carbon [37]. The loss of substrate after a certain period indicates soil fauna feeding activity, while soil flora (e.g., bacteria, fungi, etc.) play a minor role [38–40].

In trial one, concurrently with the first harvest (six weeks after planting/fertilization), three diametrical Bait-lamina strips were inserted into each pot (replicate) with the uppermost hole below the soil surface for assessing the early effects of fertilization on soil fauna feeding activity [37]. In addition, another set of strips was inserted 21 weeks after planting/fertilization to determine the late effects. In trial two, the strips were inserted into the soil after the harvest (eight weeks after planting/fertilization). Plant growth and irrigation continued as usual for the whole period that strips were in the soil. The strips were exposed to soil fauna feeding activity for seven weeks on each occasion before the strips were removed and visually inspected for the loss of the bait substrate [37]. The loss of substrate in any hole per strip was scored as empty (1), partly empty (0.5), or filled (0) [9]. Every unfilled hole (score 1) was equal to 6.25% feeding activity at any defined depth.

2.4. Statistical Analyzes

The data were registered and cleaned. The differences in yield and soil fauna feeding activity were analyzed using Minitab 20 statistical software (© 2021 Minitab, LLC (State College, PA, USA)). A one-way ANOVA test was used to evaluate the differences between fertilizing treatments. In addition, Games–Howell pairwise comparison was used to compare and group differences between treatments and plot data at a 95% confidence interval for the means. Individual standard deviations are used to estimate the confidence intervals.

3. Results

3.1. Dry Matter Yields

Dry matter (DM) yield in trial one reflects the N content provided in fertilization, and a significant difference ($p = 0.001$) was observed between the different fertilizing treatments (Figure 1A, Table S1). Summing up the DM yields from both first and second harvests showed that the highest amount of mineral fertilizer (235 kg N ha^{-1}) produced between 12–46% more yields than other fertilization treatments and 81% more yields than no fertilizer. Moreover, NEO yielded at a higher level than untreated manure and in the same range as 175 kg N ha^{-1} in mineral fertilizer, which was the same N content as in the NEO. It was also evident that no fertilizer had the lowest DM yields (Figure 1A, Table S1).

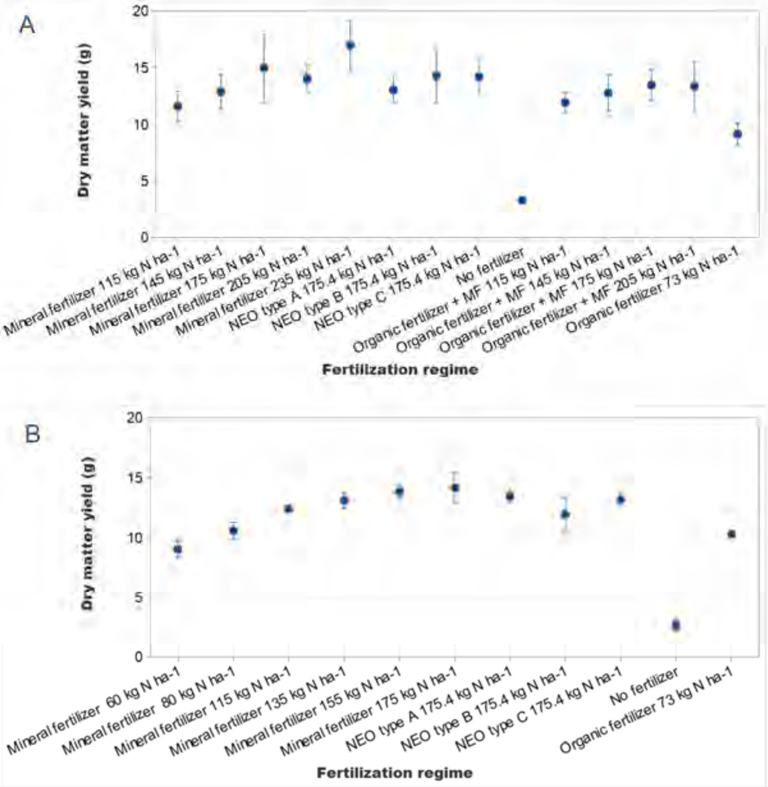


Figure 1. Effects of different fertilizing treatments on ryegrass dry matter yields (g) in trial one (**A**) and trial two (**B**). Individual standard deviations at 95% confidence interval are used in the graphs.

Similar to trial one, in trial two, the N amount in the fertilizer significantly affected the DM yields ($p = 0.001$) (Figure 1B, Table S1). Mineral fertilizer 175 kg N ha^{-1} and 155 kg N ha^{-1} had higher DM yields than the other treatments. Mineral fertilizer 175 kg N ha^{-1} produced between 3 and 63% more yield than other fertilization treatments and 81% more than no fertilizer. The different NEO fertilizers produced in the same range as mineral fertilizers with 115–155 kg N ha^{-1} while mineral fertilizer 80 kg N ha^{-1}, organic fertilizer 73 kg N/ha^{-1}, and mineral fertilizer 60 kg N ha^{-1} had lower DM yields than the other treatments with no fertilizer in the bottom (Figure 1B, Table S1).

3.2. *Soil Fauna Feeding Activity*

In trial one, soil fauna feeding activity was assessed for early and late fertilization effects. There was a significant difference between different fertilizing treatments ($p = 0.001$) regarding the early effects in trial one. Mineral fertilizer 205 kg N ha^{-1} had a higher soil fauna feeding activity than mineral fertilizer 115 kg N ha^{-1}, NEO type B 175.4 kg N ha^{-1}, and NEO type C 175.4 kg N ha^{-1} (Figure 2A, Table S1).

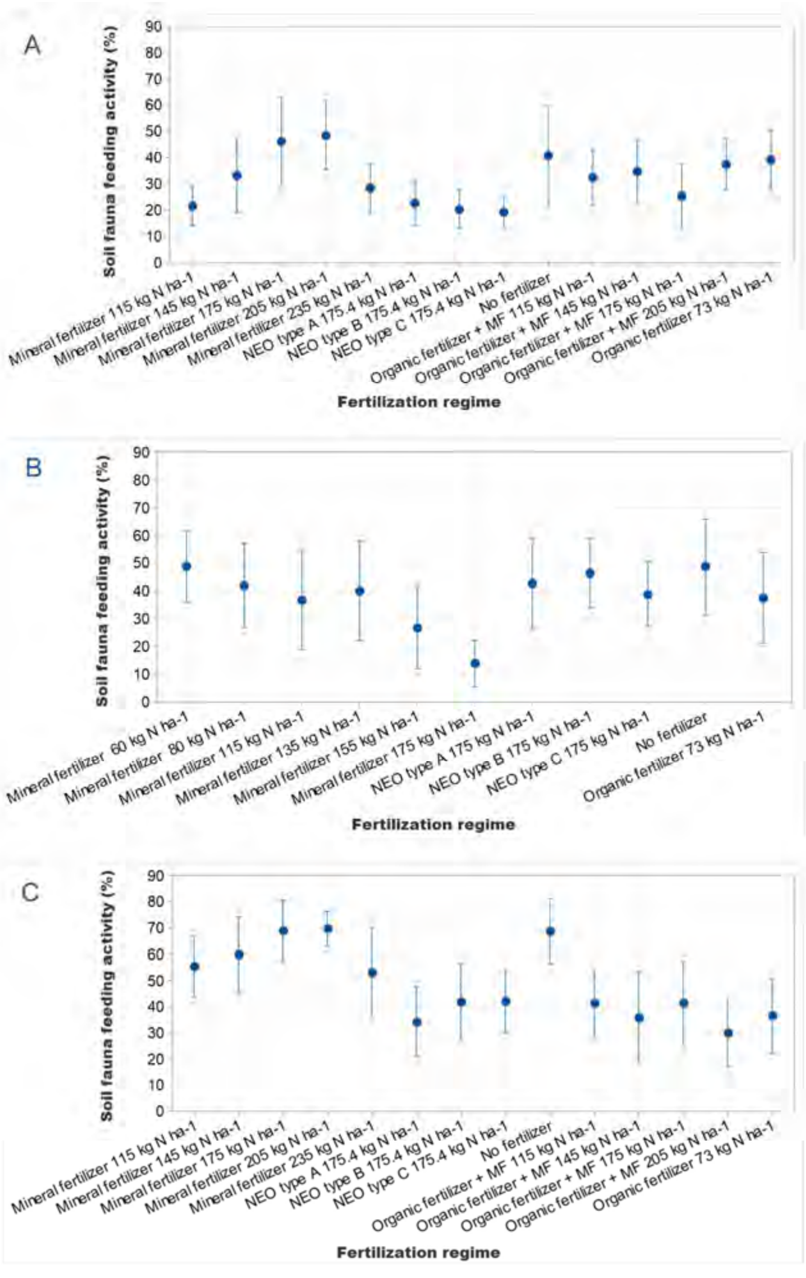


Figure 2. Effects of different fertilizing treatments on soil fauna feeding activity (%) in the early effect measurement in trial one (**A**) and trial two (**B**), and in the late effect measurement in trial one (**C**). Individual standard deviations at 95% confidence interval are used in the graphs.

Also in trial two, there was a significant difference between different fertilizing treatments ($p = 0.001$) regarding the early effects. Here, no fertilizer, NEO type B 175.4 kg N ha^{-1}, and mineral fertilizer 60 kg N ha^{-1} had higher soil fauna feeding activity than other treatments. However, mineral fertilizer 175 kg N ha^{-1} had the lowest soil fauna feeding activity (Figure 2B, Table S1).

Regarding the late effects of fertilizers on soil fauna feeding activity in trial one, likewise the early effects, there was a significant difference between different fertilizing treatments ($p = 0.001$). Mineral fertilizer 205 kg N ha^{-1} had a higher soil fauna feeding activity than organic fertilizer 73 kg N ha^{-1}, all types of NEO 175.4 kg N ha^{-1}, and all combinations of organic fertilizer + mineral fertilizer. NEO type A 175.4 kg N ha^{-1} and organic fertilizer + mineral fertilizer 205 kg N ha^{-1} had the lowest soil fauna feeding activity (Figure 2C, Table S1).

We identified a pattern where the highest feeding activity occurred in depth below 5 cm from the soil surface. In trial one, the feeding activity was at its lowest at 2 cm depth ($p = 0.001$). In trial two, there was no significant difference in the depth of feeding activity ($p = 0.08$). Both these results refer to the early effects after fertilization. The same pattern was observed for the late fertilization effects, but the differences were not significant ($p = 0.37$) (Figure 3A–C, Table S1).

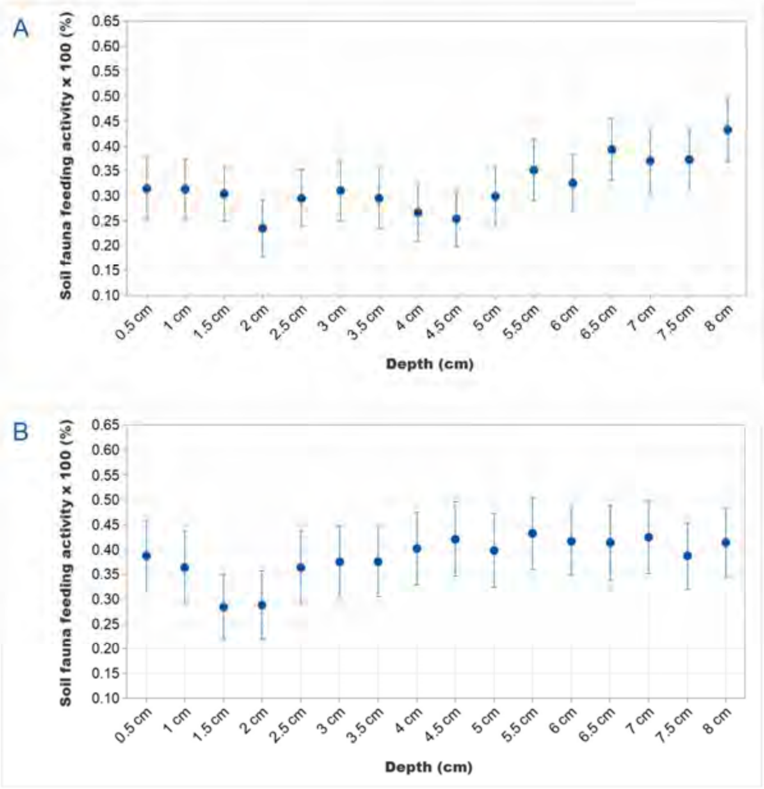


Figure 3. *Cont.*

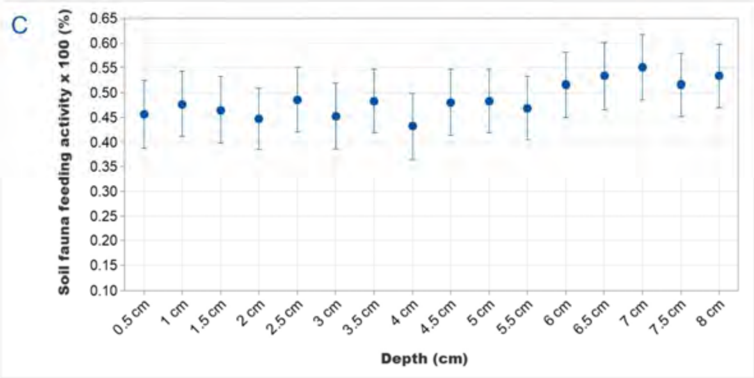


Figure 3. Effects of different fertilizing treatments on the depth of soil fauna feeding activity (×100%) in the early effect measurement in trial one (**A**) and trial two (**B**), and the late effect measurement in trial one (**C**). Individual standard deviations at 95% confidence interval are used in the graphs.

4. Discussion

NEO is a newly patented nitrogen enriched organic fertilizer and is considered a sustainable product. However, its effect on plant yield and soil living organisms has not yet been examined. Here, we investigated the alteration of ryegrass yields and soil fauna feeding activity on a sandy clay loam where NEO was compared to mineral and organic fertilizers under different fertilizing levels under controlled conditions in pot experiments.

Overall, our results showed, as expected, a positive effect of nitrogen on ryegrass yields where the yield increased with increased mineral fertilization. The N ladder in mineral fertilizer application was designed to judge the fertilization value of NEO. Application of NEO with 175 kg N ha^{-1} yielded in the same range or a little lower than mineral fertilizers with the same N content. This was a positive result for NEO. An organic N fraction can explain the slightly lower yield in the NEO (like in manure), which needs to be mineralized before it is available for plants. Furthermore, we showed that NEO produced 31–36% higher yields than non-treated manure. There were no differences in yields among the three NEO types, even though they differed in the percentage of nitrite and nitrate but with the same total N content.

The positive effect of mineral nitrogen and ryegrass yield has been documented before [6–8]. We used a sandy clay loam and found the highest yields at our maximum level of 235 kg N ha^{-1} in trial one and 175 kg N ha^{-1} in trial two. A similar result was found in field trials on a clay loam in South Africa with the highest yields at 240 kg N ha^{-1} [8], which was the maximum fertilization level in that study. The same result was reported on clay soils in Turkey [8]. Harris et al. [6] demonstrated increasing yields up to 400 kg N ha^{-1} on different loam soils in New Zealand, which is a much higher nitrogen level than we used. We could not detect a superior effect of mineral and organic fertilizer combined, as described by others [5].

While the relationship between fertilization and plant yields is well established, the knowledge about its impacts on soil-living communities requires improvements [41], and especially the knowledge on NEO as a new product is very limited. In the early effect measurement (with the Bait-lamina strips put into the soil six weeks after fertilization), fertilizers in general tend to diminish the soil fauna's feeding activity more than no fertilizer. However, there was no indication of any severe adverse impacts on the soil fauna feeding activity. This is exemplified in trial one, where mineral fertilizers with 175 kg N ha^{-1} imposed higher soil fauna feeding activity than no fertilizer. Nevertheless, this phenomenon was not verified in trial two. In trial one, the treatment with the lowest amount of mineral fertilizer (115 kg N ha^{-1}) and NEO types B and C had the lowest soil fauna feeding activity in early effect measurement, but this pattern was not confirmed in trial two. Instead, in trial two, all NEO types, organic fertilizer, and the lowest amounts of mineral fertilizer (60 and 80 kg N ha^{-1}) had better or similar soil fauna feeding activity as the highest amounts of mineral fertilizer. Taken together, all fertilizing treatments, NEO included, showed a trend with lower feeding activity compared to no fertilizer. The exceptions to this trend were mineral fertilizer 175 kg N ha^{-1} and mineral fertilizer 205 kg N ha^{-1} in trial one. Regarding the late effects measured after 21 weeks, fertilization diminished soil fauna feeding activity compared to no fertilizer regardless of fertilizer type. Again, mineral fertilizer 175 kg N ha^{-1} and 205 kg N ha^{-1} were exceptions to this trend, and organic fertilizer, all organic fertilizer + mineral fertilizer combinations, and all types of NEO showed a lower percentage of soil fauna feeding activity than no fertilizer. Thus, we see a pattern where fertilization, including NEO, tends to decrease the soil fauna feeding activity. Other studies also indicate adverse effects of mineral fertilizers on soil fauna, validating this argument [9,42–44]. However, the other valid argument is increased food availability for the soil fauna through fertilizers, whereas the soil fauna has no interest in consuming bait material in the strips. Contrasting the outcomes of this study, field studies have indicated positive effects of mineral fertilizer on soil fauna, presumably through increasing nutrient availability and enhancing plant productivity [45,46], and organic fertilizer through their advantages for plant production systems [47,48]. Therefore, we argue that other factors than fertilization alone may explain our result and affect the result. Considering that the observed tendencies partly contradict, we could conclude that there is neither a positive nor a negative effect of the fertilizing treatment on soil fauna feeding activity. One last outcome was that the soil fauna feeding activity was higher in the late measurement (21 weeks after fertilization) than in the early measurement (six weeks after fertilization). Therefore, it is valid to argue that with time, plants' root network is expanding in the soil, providing pathways for more convenient movement of soil fauna [49]. Developing a rhizosphere is a time-demanding process, especially in a closed system such as a pot experiment. We also saw that the feeding activity increased in depth below 5 cm from the soil surface. This conflicts with the finding of most field studies, where the most soil fauna feeding activity takes place in the top 5 cm of soil surface [34]. However, arguably in a pot trial where the irrigation occurs over the soil surface, nutrients and the plant roots accumulate in the pot's bottom. It is also feasible to interpret that we mixed fertilizers with the second soil layer from the bottom of the pot, which induced more feeding activity within this layer. These arguments are presumably the driving forces for more soil fauna feeding activity at this depth.

5. Conclusions

Nitrogen Enriched Organic fertilizer (NEO) produced ryegrass yields in the same range as mineral fertilizer with similar total nitrogen content, and NEO produced clearly higher yields than untreated organic manure. There was no correlation between yield data and soil fauna feeding activity. NEO, as the other fertilizer treatments, had some negative effects on soil fauna feeding activity compared to no fertilizer that tends to have the highest feeding activity. The variation was, however, big and factors other than fertilization alone seem to influence the soil fauna feeding activity. Alternatively, the Bait-lamina method is not applicable for this type of pot experiment under controlled conditions. More research is needed to clarify these uncertainties. Therefore, as the next step, we are progressing with field trials and biochemical analyses of the soil samples to investigate the effects of NEO on plant yield and soil organisms under field conditions.

Supplementary Materials: Supporting information can be downloaded from https://www.mdpi.com/article/10.3390/su14042005/s1, Table S1: Effects of different fertilizing regimes including different amounts of mineral fertilizer, three types of NEO, organic fertilizer (untreated cattle slurry), organic fertilizer + different amounts of mineral fertilizer (MF), and no fertilizer on ryegrass dry matter yields (g) in trial one (A) and trial two (B), soil fauna feeding activity (%) in the early effects in trial one (C) and trial two (D), and in the late effects in trial one (E), and the depth of feeding activity

(%) in the short term in trial one (F) and trial two (G), and in long term trial one (H). Games-Howell pairwise comparison method at 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

Author Contributions: Conceptualization, H.M. and T.C.; methodology, H.M. and T.C.; validation, H.M. and S.Ø.S.; formal analysis, H.M.; investigation, H.M. and T.C.; lab work and data acquisition: H.M. and G.H.; data curation, H.M.; writing—original draft preparation, H.M. and S.Ø.S.; writing—review and editing, H.M., S.Ø.S. and T.C.; visualization, H.M.; supervision, S.Ø.S.; project administration, T.C.; funding acquisition, T.C. and S.Ø.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Research Council of Norway.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is accessible at https://osf.io/deukp/?view_only=ecb72edabb424f5cb38c11a40773d012 (accessed on 30 January 2022).

Acknowledgments: The authors would like to thank Morten Tofastrud and Elisabeth Røe at Inland Norway University of Applied Sciences for support in project administration, and in addition, we would like to acknowledge the project's funders.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. Fertilizers Indicators. FAOSTAT 2022. Rome, Italy. Available online: https://www.fao.org/faostat/en/#data/EF (accessed on 30 January 2022).
2. Matson, P.A.; Parton, W.J.; Power, A.G.; Swift, M.J. Agricultural intensification and ecosystem properties. *Science* **1997**, *277*, 504–509. [CrossRef] [PubMed]
3. Lu, C.; Tian, H. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data* **2017**, *9*, 181–192. [CrossRef]
4. Savci, S. Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia* **2012**, *1*, 287–292. [CrossRef]
5. Körschens, M.; Albert, E.; Armbruster, M.; Barkusky, D.; Baumecker, M.; Behle-Schalk, L.; Bischoff, R.; Čergan, Z.; Ellmer, F.; Herbst, F.; et al. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: Results from 20 European long-term field experiments of the twenty-first century. *Arch. Agron. Soil Sci.* **2013**, *59*, 1017–1040. [CrossRef]
6. Harris, S.L.; Thom, E.R.; Clark, D.A. Effect of high rates of nitrogen fertiliser on perennial ryegrass growth and morphology in grazed dairy pasture in northern New Zealand. *New Zealand J. Agric. Res.* **1996**, *39*, 159–169. [CrossRef]
7. Cinar, S.; Özkurt, M.; Cetin, R. Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (Lolium multiflorum l.) in central black sea climatic zone in turkey. *Appl. Ecol. Environ. Res.* **2020**, *18*, 417–432. [CrossRef]
8. Abraha, A.B.; Truter, W.F.; Annandale, J.G.; Fessehazion, M.K. Forage yield and quality response of annual ryegrass (Lolium multiflorum) to different water and nitrogen levels. *Afr. J. Range Forage Sci.* **2015**, *32*, 125–131. [CrossRef]
9. Siebert, J.; Sunnemann, M.; Auge, H.; Berger, S.; Cesarz, S.; Ciobanu, M.; Guerrero-Ramírez, N.R.; Eisenhauer, N. The effects of drought and nutrient addition on soil organisms vary across taxonomic groups, but are constant across seasons. *Sci. Rep.* **2019**, *9*, 639. [CrossRef]
10. Bünemann, E.K.; Schwenke, G.D.; Van Zwieten, L. Impact of agricultural inputs on soil organisms—A review. *Soil Res.* **2006**, *44*, 379–406. [CrossRef]
11. Bell, C.W.; Asao, S.; Calderon, F.; Wolk, B.; Wallenstein, M.D. Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biol. Biochem.* **2015**, *85*, 170–182. [CrossRef]
12. Yuan, J.; Zhao, M.; Li, R.; Huang, Q.; Rensing, C.; Shen, Q. Lipopeptides produced by B. amyloliquefaciens NJN-6 altered the soil fungal community and non-ribosomal peptides genes harboring microbial community. *Appl. Soil Ecol.* **2017**, *117–118*, 96–105. [CrossRef]
13. Känkänen, H.; Eriksson, C. Effects of undersown crops on soil mineral N and grain yield of spring barley. *Eur. J. Agron.* **2007**, *27*, 25–34. [CrossRef]
14. Aronsson, H.; Hansen, E.M.; Thomsen, I.K.; Liu, J.; Øgaard, A.F.; Känkänen, H.; Ulén, B. The ability of cover crops to reduce nitrogen and phosphorus losses from arable land in southern Scandinavia and Finland. *J. Soil Water Conserv.* **2016**, *71*, 41. [CrossRef]
15. Karlsson-Strese, E.M.; Umaerus, M.; Rydberg, I. Strategy for Catch Crop Development: I. Hypothetical Ideotype and Screening of Species. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **1996**, *46*, 106–111. [CrossRef]

16. Jung, G.A.; Van Wijk, A.J.P.; Hunt, W.F.; Watson, C.E. Ryegrasses. In *Cool-Season Forage Grasses*; American Society of Agronomy, Inc.: Madison, WI, USA, 1996; pp. 605–641.
17. N2Applied. Nitrogen Enriched Organic Fertiliser. Available online: https://n2applied.com/ (accessed on 24 August 2021).
18. Graves, D.B.; Bakken, L.B.; Jensen, M.B.; Ingels, R. Plasma Activated Organic Fertilizer. *Plasma Chem. Plasma Processing* **2019**, *39*, 1–19. [CrossRef]
19. Ingels, R.; Graves, D. Improving Organic Fertilizer and Nitrogen Use Efficiency via Air Plasma and Distributed Renewable Energy. *Plasma Med.* **2016**, 5. [CrossRef]
20. McLaughlin, A.; Mineau, P. The impact of agricultural practices on biodiversity. *Agric. Ecosyst. Environ.* **1995**, *55*, 201–212. [CrossRef]
21. Geisseler, D.; Scow, K.M. Long-term effects of mineral fertilizers on soil microorganisms—A review. *Soil Biol. Biochem.* **2014**, *75*, 54–63. [CrossRef]
22. Wahyuningsih, R.; Marchand, L.; Pujianto, S.; Caliman, J. Impact of inorganic fertilizer to soil biological activity in an oil palm plantation. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *336*, 012017. [CrossRef]
23. Graenitz, J.; Bauer, R. The effect of fertilization and crop rotation on biological activity in a 90 year long-term experiment. *Bodenkultur* **2000**, *51*, 99–105.
24. Wei, M.; Hu, G.; Wang, H.; Bai, E.; Lou, Y.; Zhang, A.; Zhuge, Y. 35 years of manure and chemical fertilizer application alters soil microbial community composition in a Fluvo-aquic soil in Northern China. *Eur. J. Soil Biol.* **2017**, *82*, 27–34. [CrossRef]
25. Pelosi, C.; Boros, G.; van Oort, F.; Schmidt, O. Soil Oligochaeta communities after 9 decades of continuous fertilization in a bare fallow experiment. *Soil Org.* **2020**, *92*, 129–141. [CrossRef]
26. Silva, D.M.d.; Jacques, R.J.S.; Silva, D.A.A.d.; Santana, N.A.; Vogelmann, E.; Eckhardt, D.P.; Antoniolli, Z.I. Effects of pig slurry application on the diversity and activity of soil biota in pasture areas. *Ciência Rural* **2016**, *46*, 1756–1763. [CrossRef]
27. Yara. YaraMila™. Available online: https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaramila/ (accessed on 30 August 2021).
28. Yara. YaraLiva™. Available online: https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaraliva/ (accessed on 30 August 2021).
29. Lighting, L. ATS300W Specs Sheet. Available online: https://lumatek-lighting.com/lumatek-ats-300w/ (accessed on 30 January 2022).
30. Yiotis, C.; McElwain, J.C.; Osborne, B.A. Enhancing the productivity of ryegrass at elevated CO2 is dependent on tillering and leaf area development rather than leaf-level photosynthesis. *J. Exp. Bot.* **2020**, *72*, 1962–1977. [CrossRef] [PubMed]
31. Beecher, M.; Boland, T.M.; O'Donovan, M.; Eva Lewis, E. Comparing drying protocols for perennial ryegrass samples in preparation for chemical analysis. In Proceedings of the 22nd International Grassland Congress, Sydney, Australia, 13–15 September 2013; pp. 639–642.
32. TerraProtecta. The Bait-Lamina Test. Available online: http://www.terra-protecta.de/en/bait_strips.html (accessed on 24 August 2021).
33. Törne, E.v. Assessing feeding activities of soil-living animals. Bait-lamina-tests. *Pedobiologia* **1990**, *34*, 89–101.
34. Hamel, C.; Schellenberg, M.P.; Hanson, K.; Wang, H. Evaluation of the "bait-lamina test" to assess soil microfauna feeding activity in mixed grassland. *Appl. Soil Ecol.* **2007**, *36*, 199–204. [CrossRef]
35. Kratz, W. Bait-lamina test, general aspect, applications and prespectives. *Environ. Sci. Pollut. Res.* **1998**, *5*, 3.
36. Jänsch, S.; Scheffczyk, A.; Römbke, J. The bait-lamina earthworm test: A possible addition to the chronic earthworm toxicity test? *Euro-Mediterr. J. Environ. Integr.* **2017**, *2*, 5. [CrossRef]
37. ISO18311(E). Soil Quality—Method for Testing Effects of Soil Contaminants on the Feeding Activity of Soil Dwelling Organisms in the Field—Bait-Lamina Test" for Approval for the Next Stage (FDIS) until 2015-04-17 (see Recommendation Paris-2). 2015. Available online: https://www.iso.org/obp/ui/es/#iso:std:iso:18311:ed-1:v1:en (accessed on 24 August 2021).
38. Gardi, C.; Montanarella, L.; Arrouays, D.; Bispo, A.; Lemanceau, P.; Jolivet, C.; Mulder, C.; Ranjard, L.; Römbke, J.; Rutgers, M.; et al. Soil biodiversity monitoring in Europe: Ongoing activities and challenges. *Eur. J. Soil Sci.* **2009**, *60*, 807–819. [CrossRef]
39. Simpson, J.E.; Slade, E.; Riutta, T.; Taylor, M.E. Factors Affecting Soil Fauna Feeding Activity in a Fragmented Lowland Temperate Deciduous Woodland. *PLoS ONE* **2012**, *7*, e29616. [CrossRef]
40. Rożen, A.; Sobczyk, Ł.; Liszka, K.; Weiner, J. Soil faunal activity as measured by the bait-lamina test in monocultures of 14 tree species in the Siemianice common-garden experiment, Poland. *Appl. Soil Ecol.* **2010**, *45*, 160–167. [CrossRef]
41. FAO. *State of Knowledge of Soil Biodiversity—Status, Challenges and Potentialities*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2020.
42. Tao, H.-H.; Slade, E.M.; Willis, K.J.; Caliman, J.-P.; Snaddon, J.L. Effects of soil management practices on soil fauna feeding activity in an Indonesian oil palm plantation. *Agric. Ecosyst. Environ.* **2016**, *218*, 133–140. [CrossRef]
43. Birkhofer, K.; Bezemer, T.M.; Bloem, J.; Bonkowski, M.; Christensen, S.; Dubois, D.; Ekelund, F.; Fließbach, A.; Gunst, L.; Hedlund, K.; et al. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. *Soil Biol. Biochem.* **2008**, *40*, 2297–2308. [CrossRef]
44. Sánchez-Moreno, S.; Ferris, H. Suppressive service of the soil food web: Effects of environmental management. *Agric. Ecosyst. Environ.* **2007**, *119*, 75–87. [CrossRef]
45. Kosolpova, A.; Yamaltdinova, V.; Mitrofanova, E.; Fomin, D.; Teterlev, I. Biological activity of soil depending on fertilizer systems. *Bulg. J. Agric. Sci.* **2016**, *22*, 921–926.

46. van der Wal, A.; Geerts, R.H.E.M.; Korevaar, H.; Schouten, A.J.; op Akkerhuis, G.A.J.M.J.; Rutgers, M.; Mulder, C. Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands. *Biol. Fertil. Soils* **2009**, *45*, 663–667. [CrossRef]
47. Wang, S.; Chen, H.Y.H.; Tan, Y.; Fan, H.; Ruan, H. Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. *Sci. Rep.* **2016**, *6*, 20816. [CrossRef] [PubMed]
48. Sandor, M.; Brad, T.; Maxim, A.; Sandor, V.; Onica, B. The Effect of Fertilizer Regime on Soil Fauna. *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca* **2016**, *73*, 2. [CrossRef]
49. Briones, M.J.I. The Serendipitous Value of Soil Fauna in Ecosystem Functioning: The Unexplained Explained. *Front. Environ. Sci.* **2018**, *6*, 149. [CrossRef]

Supporting Information:

Mousavi, H.; Cottis, T.; Hoff, G.; Solberg, S.Ø. Nitrogen Enriched Organic Fertilizer (NEO) and its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions.

Table S1. Effects of different fertilizing regimes including different amounts of mineral fertilizer, three types of NEO, organic fertilizer (untreated cattle slurry), organic fertilizer + different amounts of mineral fertilizer (MF), and no fertilizer on ryegrass dry matter yields (g) in trial one, and trial two, soil fauna feeding activity (%) in the early effects in trial one, and trial two, and in the late effects in trial one, and the depth of feeding activity (%) in the short term in trial one, and trial two, and in long term trial one, respectively. Games-Howell pairwise comparison method at 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

Ryegrass dry matter yields in trial one	**Yield (g)**	**Grouping**
Mineral fertilizer 235 kg N ha-1	16.9	A
Mineral fertilizer 175 kg N ha-1	14.9	AB
NEO type B 175.4 kg N ha-1	14.2	AB
NEO type C 175.4 kg N ha-1	14.1	AB
Mineral fertilizer 205 kg N ha-1	14	AB
Organic fertilizer + MF 175 kg N ha-1	13.4	AB
Organic fertilizer + MF 205 kg N ha-1	13.3	AB
NEO type A 175.4 kg N ha-1	13.1	AB
Mineral fertilizer 145 kg N ha-1	12.8	AB
Organic fertilizer + MF 145 kg N ha-1	12.7	AB
Organic fertilizer + MF 115 kg N ha-1	11.9	B
Mineral fertilizer 115 kg N ha-1	11.6	B
Organic fertilizer 73 kg N ha-1	9.1	B
No fertilizer	3.2	C
Ryegrass dry matter yields in trial two	**Yield (g)**	**Grouping**
Mineral fertilizer 175 kg N ha-1	14.1	A
Mineral fertilizer 155 kg N ha-1	13.8	A
NEO type A 175.4 kg N ha-1	13.4	A
NEO type C 175.4 kg N ha-1	13.2	A
Mineral fertilizer 135 kg N ha-1	13.1	A
Mineral fertilizer 115 kg N ha-1	12.4	A
NEO type B 175.4 kg N ha-1	11.9	AB
Mineral fertilizer 80 kg N ha-1	10.6	B

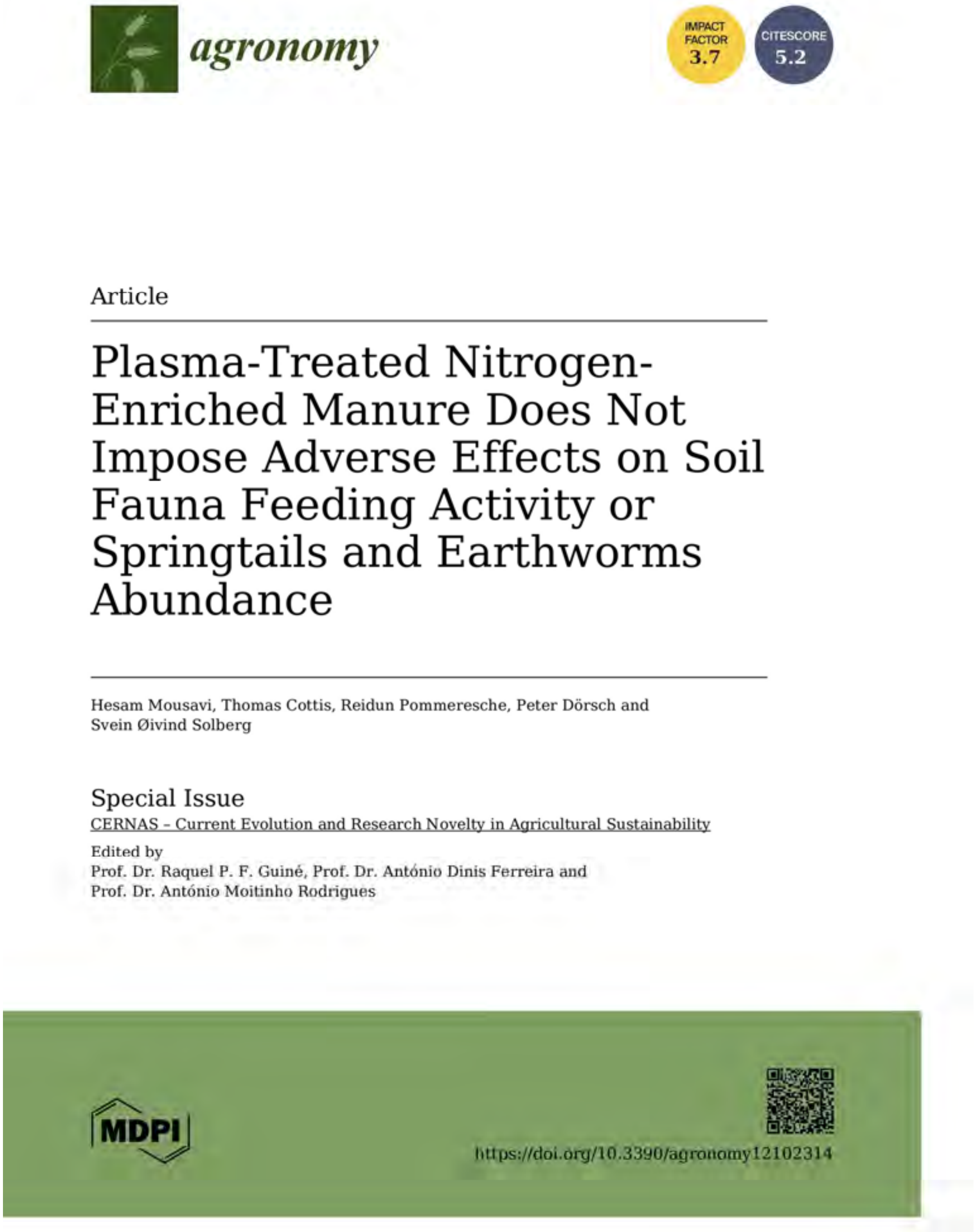
Organic fertilizer 73 kg N ha-1	10.3	BC
Mineral fertilizer 60 kg N ha-1	9.1	C
No fertilizer	2.7	D
Soil fauna feeding activity, early effects in trial one	**Feeding activity (%)**	**Grouping**
Mineral fertilizer 205 kg N ha-1	48.4	A
Mineral fertilizer 175 kg N ha-1	46	AB
No fertilizer	40.6	AB
Organic fertilizer 73 kg N ha-1	39.1	AB
Organic fertilizer + MF 205 kg N ha-1	37.2	AB
Organic fertilizer + MF 145 kg N ha-1	34.6	AB
Mineral fertilizer 145 kg N ha-1	33.1	AB
Organic fertilizer + MF 115 kg N ha-1	32.2	AB
Mineral fertilizer 235 kg N ha-1	28.1	AB
Organic fertilizer + MF 175 kg N ha-1	25.2	AB
NEO type A 175.4 kg N ha-1	22.6	AB
Mineral fertilizer 115 kg N ha-1	21.6	B
NEO type B 175.4 kg N ha-1	20.3	B
NEO type C 175.4 kg N ha-1	19.2	B
Soil fauna feeding activity, early effects in trial two	**Feeding activity (%)**	**Grouping**
No fertilizer	48.7	A
Mineral fertilizer 60 kg N ha-1	48.7	A
NEO type B 175 kg N ha-1	46.3	A
NEO type A 175 kg N ha-1	42.7	AB
Mineral fertilizer 80 kg N ha-1	41.9	AB
Mineral fertilizer 135 kg N ha-1	40.1	AB
NEO type C 175 kg N ha-1	38.8	AB
Organic fertilizer 73 kg N ha-1	37.5	AB
Mineral fertilizer 115 kg N ha-1	36.7	AB
Mineral fertilizer 155 kg N ha-1	26.8	AB
Mineral fertilizer 175 kg N ha-1	13.8	B
Soil fauna feeding activity, late effects in trial one	**Feeding activity (%)**	**Grouping**

Mineral fertilizer 205 kg N ha-1	69.7	A
Mineral fertilizer 175 kg N ha-1	69	AB
No fertilizer	68.7	ABC
Mineral fertilizer 145 kg N ha-1	59.9	ABCD
Mineral fertilizer 115 kg N ha-1	55.4	ABCD
Mineral fertilizer 235 kg N ha-1	53.1	ABCD
NEO type C 175.4 kg N ha-1	42.1	BCD
NEO type B 175.4 kg N ha-1	41.9	BCD
Organic fertilizer + MF 115 kg N ha-1	41.4	BCD
Organic fertilizer + MF 175 kg N ha-1	41.4	BCD
Organic fertilizer 73 kg N ha-1	36.7	CD
Organic fertilizer + MF 145 kg N ha-1	35.9	CD
NEO type A 175.4 kg N ha-1	34.3	D
Organic fertilizer + MF 205 kg N ha-1	30.1	D
Depth of feeding activity, early effects in trial one	**Feeding activity (%)**	**Grouping**
8 cm	43	A
6.5 cm	39	AB
7.5 cm	37	ABC
7 cm	36	ABC
5.5 cm	35	ABC
6 cm	32	ABC
0.5 cm	31	ABC
1 cm	31	ABC
3 cm	30	ABC
1.5 cm	30	ABC
5 cm	29	ABC
3.5 cm	29	ABC
2.5 cm	29	ABC
4 cm	26	BC
4.5 cm	25	BC
2 cm	23	C

Depth of feeding activity, early effects in trial two	**Feeding activity (%)**	**Grouping**
5.5 cm	43	A
7 cm	42	A
4.5 cm	42	A
6 cm	41	A
8 cm	41	A
6.5 cm	41	A
4 cm	40	A
5 cm	39	A
7.5 cm	38	A
0.5 cm	38	A
3.5 cm	37	A
3 cm	37	A
2.5 cm	36	A
1 cm	36	A
2 cm	28	A
1.5 cm	28	A
Depth of feeding activity, late effects in trial one	**Feeding activity (%)**	**Grouping**
7 cm	55	A
8 cm	53	A
6.5 cm	53	A
7.5 cm	51	A
6 cm	51	A
2.5 cm	48	A
5 cm	48	A
3.5 cm	48	A
4.5 cm	47	A
1 cm	47	A
5.5 cm	46	A
1.5 cm	46	A
0.5 cm	45	A

3 cm	45	A
2 cm	44	A
4 cm	43	A

2



agronomy

IMPACT FACTOR 3.7

CITESCORE 5.2

Article

Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance

Hesam Mousavi, Thomas Cottis, Reidun Pommeresche, Peter Dörsch and
Svein Øivind Solberg

Special Issue
CERNAS – Current Evolution and Research Novelty in Agricultural Sustainability

Edited by
Prof. Dr. Raquel P. F. Guiné, Prof. Dr. António Dinis Ferreira and
Prof. Dr. António Moitinho Rodrigues

MDPI

https://doi.org/10.3390/agronomy12102314

agronomy

MDPI

Article

Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance

Hesam Mousavi [1,*], Thomas Cottis [1], Reidun Pommeresche [2], Peter Dörsch [3] and Svein Øivind Solberg [1]

[1] Faculty of Applied Ecology, Agricultural Sciences, and Biotechnology, Inland Norway University of Applied Sciences, 2418 Elverum, Norway
[2] Norwegian Centre for Organic Agriculture, 6630 Tingvoll, Norway
[3] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, 1432 Aas, Norway
* Correspondence: hemousavi65@gmail.com

Abstract: Plasma treatment of animal manure is a new technology, enriching the manure with plant-available nitrogen. Therefore, the product is termed nitrogen-enriched organic fertilizer (NEO). The producer (N2 Applied) claims that NEO can be a sustainable alternative to conventional fertilizers used in agriculture. However, the effect of this product on soil-dwelling organisms is unknown. This study investigates and compares the effects of NEO on changes in soil fauna feeding activity, the abundance of springtails, and the abundance and weight of earthworms to mineral fertilizer, organic fertilizer (cattle slurry), and no fertilizer in pot and field experiments with sandy clay loam soil. Early effect evaluation (week 7) indicated influences on soil fauna feeding activity; among treatments, higher amounts of fertilizers went along with lower feeding activity, regardless of fertilizer type. However, the initial fertilizer application stimulation was transient and stabilized with time after fertilization towards mid-term (week 14) and late effect evaluations (week 21). Accordingly, differences between feeding activities were less than five percent at late effect evaluation. Similarly, none of the fertilizers used imposed adverse effects on the abundance of springtails and the abundance and weight of earthworms; these parameters were almost identical among all fertilizing treatments. After two years of application in field trials and in a pot experiment, NEO and the other used fertilizers seem not to harm the selected soil-dwelling organisms.

Keywords: sustainable agriculture; nitrogen; fertilizing; organic farming; soil fauna; NEO

Citation: Mousavi, H.; Cottis, T.; Pommeresche, R.; Dörsch, P.; Solberg, S.Ø. Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. *Agronomy* **2022**, *12*, 2314. https://doi.org/10.3390/agronomy12102314

Academic Editors: Raquel P. F. Guiné, António Dinis Ferreira and António Moitinho Rodrigues

Received: 27 August 2022
Accepted: 21 September 2022
Published: 26 September 2022

1. Introduction

The current trends of population growth and resource scarcity underline the importance of using new sustainable technologies in agriculture. Agri-food systems worldwide depend severely on mineral fertilizers [1], and current plant production systems are intensively fertilized with nitrogen (N) [2,3]. According to the FAO, the global nitrogen input into agriculture is eight times higher today than in the 1960s [4].

The favorable effect of N fertilization on plant productivity is well recognized [5–7]. However, N intensification has many severe trade-offs [3,8]. Although increased food production is crucial for sustaining an increasing human population, preserving soil fertility is also critical. While increasing food production per area is commonly highlighted, the effects of excessive fertilizing on soil organisms and their functions are often neglected [9,10]. Overuse of fertilizers can lead to air, soil, and water pollution, as well as adverse effects on biodiversity and the climate [11]. Besides, soil nutrients are manipulated through fertilization, and changes in functional soil groups are stimulated by favoring some groups over others [12]. Soil fauna and soil microorganisms contribute to various ecosystem services such as plant health, disease protection, pathogenicity, and nutrient turnover [13].

Considering the European Green Deal and the "Farm to Fork strategy," the European Commission aims for at least a 50% reduction in nutrient leaching by 2030. Accordingly, a 20% reduction in fertilizer use is anticipated [11]. Moreover, identifying fertilization regimes with the least possible adverse impact on soil organisms is fundamental as it enhances sustainability in food production. Hence, there is a necessity for the purposeful use of fertilizers, e.g., fertilizing with mineral and organic fertilizers, fertilizer efficiency enhancement [14], and developing novel high-tech fertilizers.

Nitrogen Enriched Organic fertilizer (NEO) has been introduced as a novel fertilizer with potentially advantageous properties [15]. Atmospheric nitrogen is fixed as nitrogen oxides (NOx) by a plasma process using green electricity and added to organic fertilizers (e.g., manure, slurry, digestate) using N2 Applied's (Asker, Norway) patented unit. Once the NOx reacts with water, it forms nitrous acid (HNO_2) and nitric acid (HNO_3), which lowers the pH of the slurries and stabilizes it. The units are small enough to allow farmers to produce their NEO locally, resulting in self-sufficiency and enhanced agricultural sustainability when substituting conventional organic and mineral fertilizers, nonetheless because of lowered ammonia (NH_3) and methane (CH_4) emissions [15–17]. NEO is highly fluid, holding less than 10% solid particles due to filtration during the production process. In the present study, we investigate and compare the effects of NEO on soil living organisms with those of other fertilizers.

Hypothetically, mineral fertilizers can enhance soil biological activity by increasing plant productivity and residue return [10,18]. Studies on fertilizer effects on the abundance and weight of earthworms found that a combination of mineral and organic fertilizers was even more significant than mineral fertilizer alone [19–22]. Another study indicated a positive effect of mineral fertilizer on springtails and mite abundance, despite the reduction in species richness [23]. On the contrary, N fertilizers, mainly ammonium N, can potentially contribute to diminishing soil biological activity by acidifying soil and inducing changes in soil functional communities [10,18,24,25]. Besides, repressing certain soil enzymes involved in nutrient cycles, e.g., the amidase involved in the N cycle, is likely due to the repeated application of mineral fertilizers [24].

Similarly, perhaps due to reduced plant species richness, reduced soil microbial weight was reported in perennial grassland under high N fertilizing rates. However, results from annual croplands do not support this conclusion [9,26–28], despite results being highly dependent on fertilization rates [18,21]. Regardless, the functional activity of soil organisms is a complex trait controlled by a multitude of environmental and management factors and recurrent mineral N fertilization [25]. It was shown that repeated application of organic fertilizers stimulates soil microbial and faunal growth and activity [18,19,29,30]. Indeed, organic amendments provide carbon for soil living communities and improve productivity and residue return. In addition, organic fertilizers enhance soil microbial and faunal communities more than chemical fertilizers. Albeit, this positive effect is expressed more when combining organic and mineral fertilizers [19,20,29]. However, these effects vary between annual and perennial production systems [29].

The susceptibility of soil fauna and invertebrates to elevated nitrogen levels differs. For example, soil fauna feeding activity under fertilization has been reported to be reduced in the short-term [9,24] and the long term [31]. Another study found that the abundance of springtails decreased following cattle slurry application; however, it recovered, but not entirely to initial numbers, later during the same growing season [32]. Furthermore, there are positive reports about fertilizers enhancing soil faunal structure, diversity, and feeding activity [26,27,31], specifically for springtails in the topsoil layer [33]. The mentioned controversies highlight the importance of investigating fertilizer effects on soil biota, especially when dealing with novel fertilizers such as NEO.

The current study aimed to (1) identify if NEO has any detrimental effects on soil fauna compared to conventional fertilizers and (2) develop a method for evaluating the immediate effect of fertilizers on earthworm abundance and weight. A preliminary study showed that NEO did not negatively affect soil fauna feeding activity more than other fertilizers [34]. In the present study, we expand our research using a different type of NEO and compare effects on the abundance of earthworms and springtails as "bioindicators of soil quality" [35–37]. Fertilization treatments included mineral fertilizer, NEO, untreated cattle slurry, and a combination of organic and mineral fertilizers.

2. Materials and Methods

In this study, we conducted three sets of experiments. First, a growing chamber experiment to identify and compare the fertilizer effects on soil fauna feeding activity; second, a field experiment to identify and compare fertilizer effects on the abundance of springtails. Third, an outdoor experiment to identify and compare the immediate effect of fertilization on the abundance and weight of earthworms.

2.1. Soil Fauna Feeding Activity

2.1.1. Experimental Design

We conducted pot experiments in a growing chamber at Inland Norway University of Applied Sciences. Perforated pots (13 × 18 cm^2 with 2.5 L of field soil were used. Treatments were distributed randomly among the four replicates after the pots were fertilized at loading time.

The trial consisted of five fertilization regimes distributed in seven fertilization treatments (Table 1); no fertilizer; mineral fertilizer (Yara Mila 18-3-15) [38]; NEO type D (N2 Applied) [15]; organic fertilizer (untreated cattle slurry); and organic fertilizer + mineral fertilizer (Yara Liva 16-0-0) [39]. NEO and untreated slurry were applied in liquid form, while Yara Mila and Yara Liva were pelleted. Treatments were (1) no fertilizer, (2) mineral fertilizer 73 kg N ha^{-1}, (3) mineral fertilizer 175 kg N ha^{-1}, (4) NEO type D 73 kg N ha^{-1}, (5) NEO type D 175 kg N ha^{-1}, (6) organic fertilizer 73 kg N ha^{-1}, (7) organic fertilizer + MF 175 kg N ha^{-1}.

Table 1. Fertilizing treatments and application rates used in the growing chamber trial.

	Fertilizing Treatment	Organic Fertilizer (Tons ha^{-1})	Kg N in Yara Mila18-3-15 (kg ha^{-1})	Kg N in Organic Fertilizer (kg ha^{-1})	Kg N in Yara Liva 16-0-0 (kg ha^{-1})	Total kg N (kg ha^{-1})
1	No fertilizer	-	-	-	-	0
2	Mineral fertilizer 73 kg N ha^{-1}		73			73
3	Mineral fertilizer 175 kg N ha^{-1}		175			175
4	NEO type D 73 kg N ha^{-1}	22		73		73
5	NEO type D 175 kg N ha^{-1}	50		175		175
6	Organic fertilizer 73 kg N ha^{-1}	55		73		73
7	Organic fertilizer + mineral fertilizer 175 kg N ha^{-1}	55		73	102	175

NEO type D had a pH of 5.22 and contained 1746 mg L^{-1} NH_4^+ –N, 1131 mg L^{-1} NO_2^- –N, and 1562 mg L^{-1} NO_3^- –N, totaling 4439 mg L^{-1} N. The untreated slurry had a pH of 7.32, containing 1804 mg L^{-1} NH_4^+ –N and 149 mg L^{-1} NO_2^- –N, totaling 1953 mg L^{-1} N. Therefore, we targeted a slurry amount of 55 tons ha^{-1}; nonetheless, during production, the N2 applied apparatus excludes all dry materials bigger than 5 mm; as a result, NEO's quantity decreases by 10% to 50 tons ha^{-1}. Therefore, each ton of untreated slurry contained 1.95 kg of plant-available N, while each ton of NEO contained 4.44 kg of plant-available N.

The soil was acquired from the adjacent experimental farm and analyzed at Eurofins soil lab (https://www.eurofins.no/agro-testing/ (accessed on 20 July 2022)), indicating a sandy clay loam texture, more than 10% clay, and soil organic matter of 4.5%. The soil pH was 7.4, which is relatively high, with a normal phosphorus status (P-AL = 11 mg/100 g), and a low potassium status (K-AL = 5 mg/100 g). Moreover, we estimated the soil's field capacity at 33.6% VWC, with a total pore volume of 41.4%.

In order to simulate field conditions, we planted seeds in the pots. Pots were prepared following the protocol that we developed before [34]. First, a soaked paper tissue was laid at the lowermost of the perforated pots to prevent soil outpour. Then, an initial 0.6 L (5 cm) soil load into the pots. Next, a soil load of 0.8 L (6 cm) was mixed with the fertilizer. Fertilizers were dosed following the advised field application rates (measured in tons per hectare), accounting for the soil surface in each pot (169 cm^2) (Table 1). Afterward, three rows of Italian ryegrass seeds (*Lolium multiflorum Lam.*), variety "Barpluto" (NAK Nederland/Ref. DE148-214011) per pot were then sown over the top of 0.9 L (6 cm) of additional soil. Finally, 0.2 L (1 cm) of soil was added to form the surface soil.

In the growing chamber, we used Lumatek ATS300W 80 × 80 cm LED light pads (https://lumatek-lighting.com/ (accessed on 21 July 2022)) that delivered a complete visible light spectrum (380–780 nm wavelength) recommended for plantation under controlled conditions [40]. Three adjacent LED pads were positioned 35 cm above the plants and were uplifted alongside plant growth. The light and dark intervals were adjusted according to Nordic summer days with 16 h light and 8 h darkness. Additionally, 16 pots per LED pad were confirmed to receive equal light using a digital light intensity meter. Throughout the experiment, the growth chamber had a temperature of 16 °C.

Five hundred milliliters of water, or 55% of the field capacity for our dry soil, was used to irrigate the pots at first since the soil was not entirely dry at pot preparation. After that, pots were irrigated with 200 mL of water thrice a week for the first four weeks. However, as plants progressed in the developmental stages, irrigation frequency was increased the weeks before harvest upon visual inspection [41].

The pots were positioned for the first two weeks adhering to each other. However, from week three, there was a five cm distance between the pots to avoid plants competing for light and space. Thinning was performed following germination; 24 vigorous plants per pot were kept (3 × 8 rows). Moreover, a few germinated weeds were removed by hand.

2.1.2. Evaluating Feeding Activity

Bait-lamina strips (Terra Protecta GmbH, Berlin, Germany) [42] were used to evaluate soil fauna feeding activity. This method is considered efficient, rapid, and reproducible with high statistical applicability [43,44]. This method evaluates the functional activity of soil fauna, feeding activity as one of the critical factors in soil nutrient cycling [44–46]. The technique has helped researchers screen various soil management practices and has given valuable information on the feeding activity of soil fauna. [44]. In this method, 16 1.5 mm diameter holes are located 5 mm apart on perforated PVC strips (1 mm × 6 mm × 120 mm). The holes are filled with bait substrate. The bait substrate comprises 5% activated carbon, 25% wheat bran, and 70% cellulose powder [47]. After a certain period of exposure to soil, the degree to which substrate is used up in the holes reveals the feeding activity of soil fauna, whereas soil microorganisms (e.g., bacteria, nematodes, fungi) have a negligible effect [42,48–50].

We conducted preliminary tests to determine proper intervals for the bait-lamina sampling in a pot experiment. In these tests, we noticed that after four weeks of soil fauna feeding activity, the percentage of bait consumption in the strips varied from 3–29%. Moreover, after extending the period to eight weeks, we had several strips with all holes empty, showing 100% feeding activity. Therefore, we determined seven weeks as an appropriate test period.

In this experiment, we planted three Bait-lamina strips diametrically in each pot (replicate) when watering for the first time to assess and compare the early effect of fertilization on soil fauna feeding activities [47]. Seven weeks later, the first set of strips was taken out, and the second set was inserted in the same order as the first set to evaluate the mid-term effect. Seven weeks later (week 14 after plantation/fertilizing), the second set was taken out, and the third set was inserted to evaluate the late effect of fertilizing soil fauna feeding activity. At last, this set was taken out seven weeks later (week 21 after plantation/fertilizing). Plant growth and watering were sustained until the experiment's termination to simulate the conditions seen in an actual field. The strips were visually examined for the removal of the bait substrate on each sampling [47]. Three categories—empty (1), partially empty (0.5), or filled (0)—were assessed and used to describe the disappearance of the bait substrate [9]. With a maximum of 100% feeding activity (all 16 empty holes), each empty hole (score 1) was equivalent to 6.25% feeding activity.

2.2. *The Abundance of Springtails (Collembola)*

Springtails live in all soil layers, depending on soil moisture, and have diverse life forms in different soil strata and nutrition types [51]. They graze on fungi, algae, and bacteria or feed on plant detritus or other organic substances. [52]. They are great soil bio-indicators, especially in shallow soils. As a pray for other arthropods, they play a central role in the food chain [35].

2.2.1. Experimental Design

We conducted springtail sampling in two different field trials that had been fertilized with NEO and other fertilizers one year before the first sampling. The first trial was a cereal field located at Blæstad experimental farm at Inland Norway University of Applied Sciences (60°49′11.7″ N 11°10′48.4″ E). The second trial was on a grass field located at Stjørdal, Trøndelag (63°20′33.4″ N 10°17′56.9″ E). The experimental design in both trials was a traditional randomized complete block design with four replicates. Both trials consisted of four fertilization regimes; mineral fertilizer (Yara Mila 18-3-15) [38]; NEO type B (N2 Applied) [15]; organic fertilizer (untreated cattle slurry); and no fertilizer. Both fields were fertilized for two consecutive years. The grain field was fertilized once a year: before sowing: (22 April 2020 and 27 April 2021). The grass field was fertilized twice a year: in early spring (27 April 2020 and 4 May 2021) and after the first harvest (24 June 2020 and 15 June 2021).

Fertilizer doses in the grain field were (1) mineral fertilizer 666.6 kg ha^{-1}, (2) NEO 37.6 tons ha^{-1}, (3) organic fertilizer 41 tons ha^{-1}, and (4) no fertilizer. Then again, doses in the grass field were (1) mineral fertilizer 650 kg ha^{-1} in spring + 500 kg ha^{-1} after the first harvest, (2) NEO 37.5 tons ha^{-1} + 28 tons ha^{-1} after the first harvest, (3) organic fertilizer 41 tons ha^{-1} + 30.5 tons ha^{-1} after the first harvest, and (4) no fertilizer. NEO type B had a pH of 5.35 and contained 1480 mg L^{-1} NH_4^+ –N, 777 mg L^{-1} NO_2^- –N, and 1250 mg L^{-1} NO_3^- –N, totaling 3507 mg L^{-1} N. The cattle slurry used in this experiment had a pH of 7.32, and it contained 1804 mg L^{-1} NH_4^+ –N and 149 mg L^{-1} NO_2^- –N, totaling 1953 mg L^{-1} N.

NEO and untreated slurry were applied in liquid form while mineral fertilizer was pelleted. For a homogenous liquid fertilizer, all the barrels were stirred well prior to bottling/spreading to dissolve the sediments. Next, the fertilizers were dispersed manually using containers and rapidly harrowed with the soil using a tractor before sowing in the grain field and spreading on the grass field surface. The grain field was seeded with barley 'Rødhette' (180 kg ha^{-1}) in 2020 and spring wheat 'Mirakel' (220 kg ha^{-1}) in 2021. The grass field was a mixture of timothy, meadow fescue, and red clover seeded in 2019. In the grain field, herbicides were applied once in June with Ariane S (Corteva Agriscience, Puerto Rico) and once at the end of the growing season with Roundup (Bayer, Germany). No irrigation was applied. The 2021 season at Blæstad was decent regarding cereal growth, with a relatively cool May and a little over average precipitation: 78 mm in May and 62 mm in June. Moreover, in Trøndelag, the season was good, with precipitation around normal.

The soil in the field trial at Blæstad was identical to the soil we used in the growing chamber experiment (see Section 2.2.1). The soil in the grass field trial at Trøndelag was classified as clay loam. The organic matter content was 5.1%, pH was 6,2, and plant available phosphorus and potassium were normal (P-AL = 8, K-AL = 7), but the potassium reserve was high ($KHNO_3$ = 140).

2.2.2. Evaluating the Abundance of Springtails

The soils from experimental plots in the grain and grass fields were sampled twice in 2021 for springtail abundance. The first sampling occurred on 15 June 2021, some weeks after fertilization. The second sampling was on 20 October 2021, after harvesting. The temperature on the first sampling day in June was 20 °C and 6 °C after some rainy days in October when the soil was sampled again.

Three diametric samples were collected on each replicate's corners and in the center of the field plots. First, soil sampling was conducted by hammering down a corer (5 cm high, 5 cm diameter = 98.17 cm^3 volume) in the surface soil and collecting the sample into a zipper bag using a spade [35]. The samples were transferred directly to the lab, and the three samples from each replicate were placed upside down on slightly modified Berlese funnels [35,53,54]. In this method, moisture, heat, and light gradient drive the soil organisms to move away from the heat source (60 W lamp) [55], passing a mesh screen and falling into the vessel, ending in the collection tube filled with 91% ethanol. This way, animals can be preserved for further investigation for a long time. The samples remained in extraction units for a week on each sampling occasion. Following completion of extraction, the abundance of springtails in samples was counted and registered using a light microscope. Springtail abundances were scaled up to 1 m^2 and −5 cm depth, estimating the number of soil cores fitting into 1 m^2 (169.8).

2.3. *The Fate of Earthworms (Lumbricidae)*

Earthworms are medium to large oligochaetes that are substrate feeders and play an essential role in decomposition in the soil [35]. Due to several essential functions, their activity increases soil fertility. However, their distribution highly depends on moisture, soil type, pH, and vegetation. Earthworms are categorized into three ecological groups: litter dwellers, horizontal burrowers, and deep burrowers [56]. Because of their size, a significant fraction of the biomass in loamy meadows is composed of earthworms; however, they are scarce in shallow or acid soils [57,58]. In this study, we developed a protocol for evaluating the immediate effect of fertilizers on earthworms. The earthworms used in the experiments were a mixture of juveniles and adults from the most common Norwegian earthworms: geophagous (soil eating) field worms (*Aporrectodea caliginosa*) and pink worm (*Aporrectodea rosea*). In addition, other common species in Norwegian arable soil include dew worm *Lumbricus terrestris*, *L. rubellus*, and a few individuals of the less common *Allolobophora chlorotica* [30]. The earthworms used in our trials were found in an organic vegetable garden adjacent to the experimental field.

2.3.1. Experimental Design

The experiment was repeated twice in June 2021 and June 2022, with three replicates. The study location was at Blæstad experimental farm, Innlandet (60°49′11.7″ N 11°10′48.4″ E); the soil analysis was identical to the growing chamber experiment (Section 2.1.1), and the fertilizer treatments were (1) no fertilizer, (2) mineral fertilizer (Yara Mila) 666 kg ha^{-1}, (3) NEO type B (2021), and type D (2022) 3.4 tons ha^{-1}, and (4) organic fertilizer (untreated slurry) 3.7 tons ha^{-1}. Over, NEO and mineral fertilizer contained almost equal N per hectare in both experiments. The duration of the experiments was eight days.

2.3.2. Changes in Abundance and Weight of Earthworms

The developed protocol is as follows:

1. Holes with 30 cm diameter and 20 cm are dug out in the field. The soil from the holes was visually inspected to exclude present earthworms.
2. Earthworm-proof but water-permeable textile (tested before the experiment) is inserted into the hole.
3. Earthworms (Lumbricidae) used in the experiment were excavated from the same experimental farm two days before and stored in a pile of soil pending the experiment. On the day of starting the experiment, the earthworms were detached from the soil pile, sorted, weighed, and an equal number of worms (11 in the 2021 trial and 13 in the 2022 trial) making up a similar total weight were deposited in separate containers and marked. The worms were not rinsed before weighing. The worms were handled cautiously and remained detached from the soil for the shortest possible period. After counting and weighing, earthworms were transferred to other containers with soil.
4. Next, 10 cm of soil was filled back into the holes, and the earthworms were placed over the top.
5. The next 5 cm of soil was carefully mixed with the fertilizer and filled back into the hole.
6. As a supplementary food source for the worms, 100 g of grass was spread on this layer.
7. Two cm loose soil scattered over.
8. Finally, the last 3 cm of loose soil was scattered on the top.
9. Outer edges of the textile were fetched together and closed over. At last, a heavy substance (a stone) was placed over the top to inhibit wind opening or bird feeding. Finally, the experimental units were covered with white plastic tarpaulin on days of intense sun to avoid excessive temperature caused by the sun and the black textile.
10. At the end of the experiment (8 days), the soil bags were lifted out of the soil and dispersed over a flat surface. The living earthworms were carefully handpicked, counted, and weighed in less than five minutes to avoid desiccation.

2.4. Data Handling and Statistical Analyzes

We registered and sorted the data from each respective experiment. Using Minitab 20 statistical software (2021 Minitab, LLC (State College, PA, USA)), the differences in soil fauna feeding activity, the abundance of springtails, and variations in weight and abundance of earthworms were examined. The differences among fertilizing treatments were assessed using a one-way ANOVA and Welch's test. Games–Howell pairwise comparison was further utilized to compare, categorize, and plot data at a 95% confidence interval for the means. The error bars in the graphs are calculated by using individual standard deviations.

3. Results

3.1. Soil Fauna Feeding Activity

Using a growth chamber experiment where all variables except fertilization were held constant, we examined and evaluated the impact of different fertilization treatments on the feeding activity of soil fauna. We investigated the early effects (seven weeks), mid-term effects (14 weeks), and late effects (21 weeks).

There was a significant early fertilization effect on the feeding activity ($p = 0.001$). However, this effect was not associated with the type of fertilizer but with the amount of fertilizer applied (Figure 1A, Table S1). After seven weeks, mineral fertilizer 73 kg N ha^{-1} (78.13%), organic fertilizer 73 kg N ha^{-1} (74.74%), NEO type D 73 kg N ha^{-1} (73.70%), and organic + mineral fertilizer 175 kg N ha^{-1} (61.98%) exhibited increased soil fauna feeding activity relative to no fertilizer (54.17%). Both NEO type D 175 kg N ha^{-1} (49.22%) and mineral fertilizer 175 kg N ha^{-1} (46.35%) tended to have lower soil fauna feeding activity than soil without fertilizer. Additionally, the lead was insignificant even though the mixture of organic and mineral fertilizers was the only high N content treatment that improved soil fauna feeding activity above that of no fertilizer (Figure 1A, Table S1).

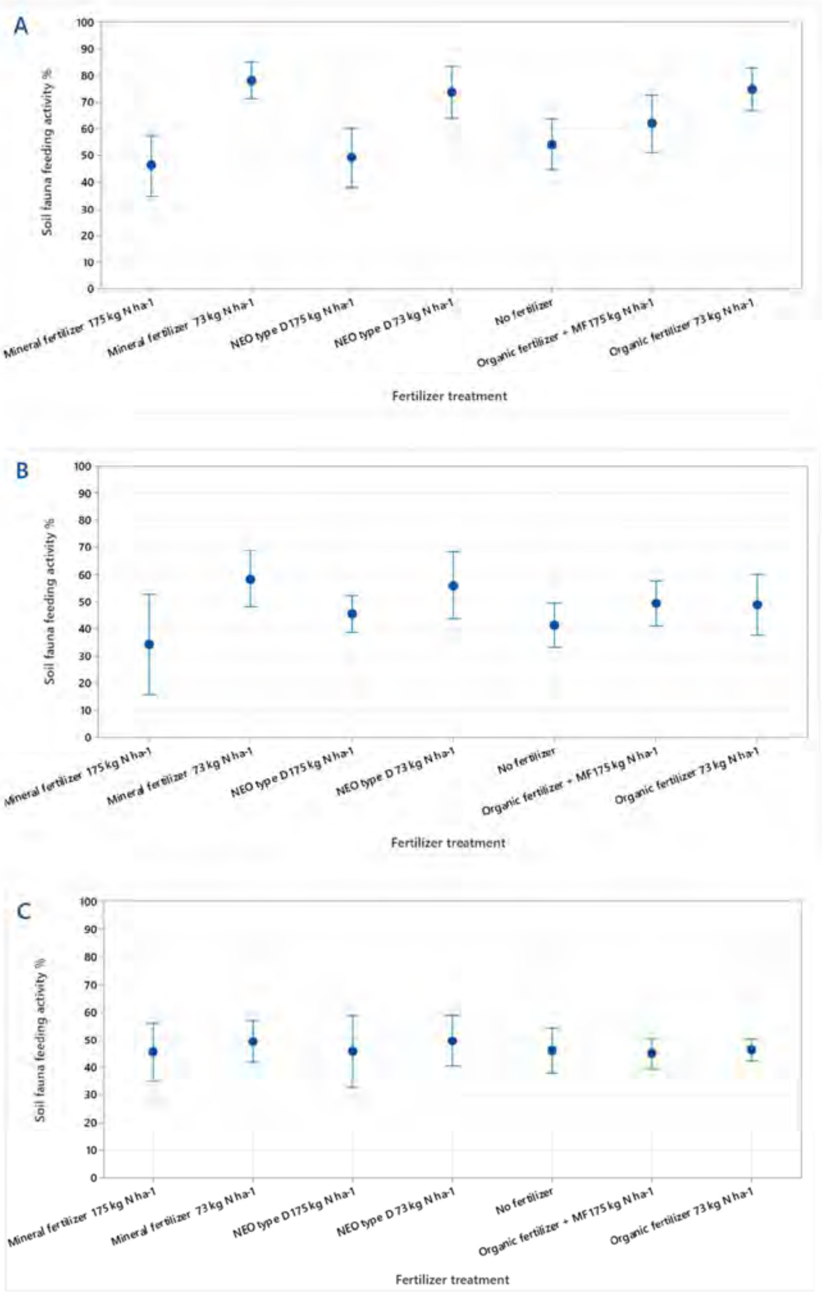


Figure 1. Effects of different fertilization treatments on soil fauna feeding activity (%) (**A**) seven weeks after fertilizing, (**B**) 14 weeks after fertilizing (7–14 weeks), and (**C**) 21 weeks after fertilizing (14–21 weeks). Error bars are individual standard deviations at a 95% confidence interval.

Regarding the mid-term effect, 14 weeks after fertilizing, initial (week 7) differences in soil faunal feeding activity converged and became more even between treatments. However, the reduction was more evident among those treatments with higher feeding activity during the initial weeks (Figures 1B and 2). Hence, the average feeding activity was 62.61% among all treatments seven weeks after fertilizing, which dropped significantly ($p = 0.001$) to 47.75% at the mid-term evaluation (Figure S1, Table S1).

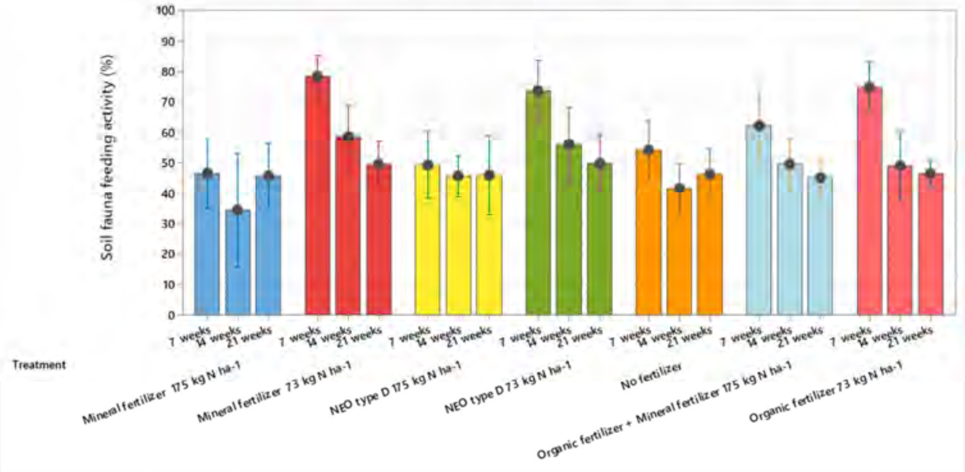


Figure 2. Early (0–7 weeks), mid-term (7–14 weeks), and late effects (14–21 weeks) on soil fauna feeding activity (%) between different fertilization treatments. Error bars are individual standard deviations at a 95% confidence interval.

Furthermore, at mid-term evaluation, feeding activities were not significantly different ($p = 0.08$) among fertilization treatments. Instead, mineral fertilizer 73 kg N ha^{-1} (58.44%), NEO type D 73 kg N ha^{-1} (55.99%), organic + mineral fertilizer 175 kg N ha^{-1} (49.48%), organic fertilizer 73 kg N ha^{-1} (48.96%), and NEO type D 175 kg N ha^{-1} (45.57%) had higher soil faunal feeding activity than no fertilizer (41.41%). By comparison, mineral fertilizer 175 kg N ha^{-1} (34.38%) had the lowest feeding activity. Thus, only the mineral fertilizer with a high N content reduced the ability of soil fauna to feed; however, as mentioned earlier, this was not statistically significant (Figure 1B, Table S1).

The late fertilization effect on feeding activity resembled the mid-term effect, i.e., despite a slight insignificant average reduction from mid-term to late effect among all fertilizing treatments (47.75% to 46.88%), soil fauna feeding activity appeared to stabilize seven weeks after fertilization without showing any significant effects (Figures 2 and S1).

The lower amounts of fertilizer, regardless of fertilizer type, supported higher feeding activity, NEO type D 73 kg N ha^{-1} (49.74%), mineral fertilizer 73 kg N ha^{-1} (49.48%), organic fertilizer 73 kg N ha^{-1} (46.35%) showed higher feeding activity than no fertilizer (46.09%). On the other hand, higher fertilizer amounts, NEO type D 175 kg N ha^{-1} (45.83%), mineral fertilizer 175 kg N ha^{-1} (45.57%), and organic + mineral fertilizer 175 kg N ha^{-1} (45.05%) had lower feeding activity. However, the difference between the highest and lowest feeding activities was a maximum of 4.2% and insignificant (Figure 1C, Table S1).

Lastly, low amounts of NEO type D, mineral fertilizer, organic fertilizer, and to some extent, the combination of organic and mineral fertilizer seemed to stimulate soil fauna feeding activity in the initial weeks after fertilization. However, this early effect gradually disappeared, whereas other treatments, including no fertilizer, had more or less constant soil faunal feeding activity throughout the experiment (Figure 2).

3.2. The Abundance of Springtails (Collembola)

We investigated and compared the effects of different fertilization treatments on the abundance of springtails at two field locations; one under cereal and another under grass cultivation. Both fields were fertilized for two consecutive years. Moreover, two samplings were performed, once just before fertilization in early summer and another during fall.

During summer, the abundance of springtails in the cereal field was slightly higher for organic fertilizer than NEO and mineral fertilizer; 509.4, 467, and 297.1 per m^2, respectively (Figure 3A, Table S1). However, the difference was insignificant (p = 0.25). The same pattern was observed during the fall. The number of springtails was slightly higher for organic fertilizer and no fertilizer than NEO and mineral fertilizer; 213.3, 213.3, 169.8, and 169.8 per m^2, respectively (Figure 3B, Table S1). Likewise, the difference between fertilization treatments in the fall sampling was insignificant (p = 0.669).

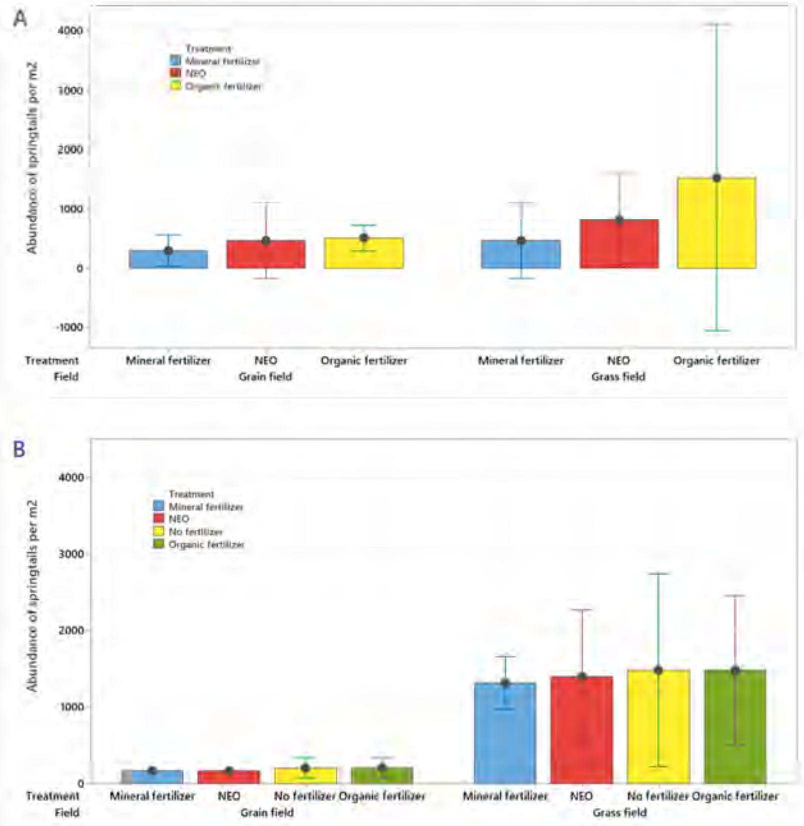


Figure 3. The effect of different fertilization treatments on the abundance of springtails per m^2 in (**A**) summer and (**B**) fall samplings. Error bars are individual standard deviations at a 95% confidence interval.

Similarly, no fertilization effects were found regarding springtail abundance in the grass field during summer and fall (p = 0.404, p = 0.943). However, during summer, higher abundance was observed for organic fertilizer than NEO and mineral fertilizer; 1528, 807, and 467 per m^2, respectively (Figure 3A, Table S1). In the fall, a slightly higher abundance was observed for organic fertilizer and no fertilizer than NEO and mineral fertilizer; 1486, 1486, 1401, and 1316, respectively (Figure 3B, Table S1).

Generally speaking, the springtail abundance was higher in the grass field during fall than in summer and almost identical in the grain field during both seasons. Nevertheless, none of the fertilizer treatments affect springtail abundance regardless of field and season.

3.3. *The Fate of Earthworms (Lumbricidae)*

In the 2021 trial, the abundance of earthworms for all treatments increased after eight days, except for the treatment with no fertilizer. However, the difference was insignificant among fertilizing treatments (p = 0.38). Organic fertilizer showed an average increase of 4.33 worms, followed by mineral fertilizer, 4, and NEO, 2.33, while no fertilizer had one fewer living earthworm than the beginning (Figure 4A, Table S1). Moreover, like for abundance, a similar pattern was observed for the average weight change. Among all captured earthworms, mineral fertilizer had an increment of 4.07 g after eight days, followed by organic fertilizer 2.87 g, NEO 1.93 g; however, the no fertilizer control had 1.67 g fewer earthworms (Figure 4A, Table S1). Nevertheless, the difference in weight change among fertilizing treatments was insignificant (p = 0.34).

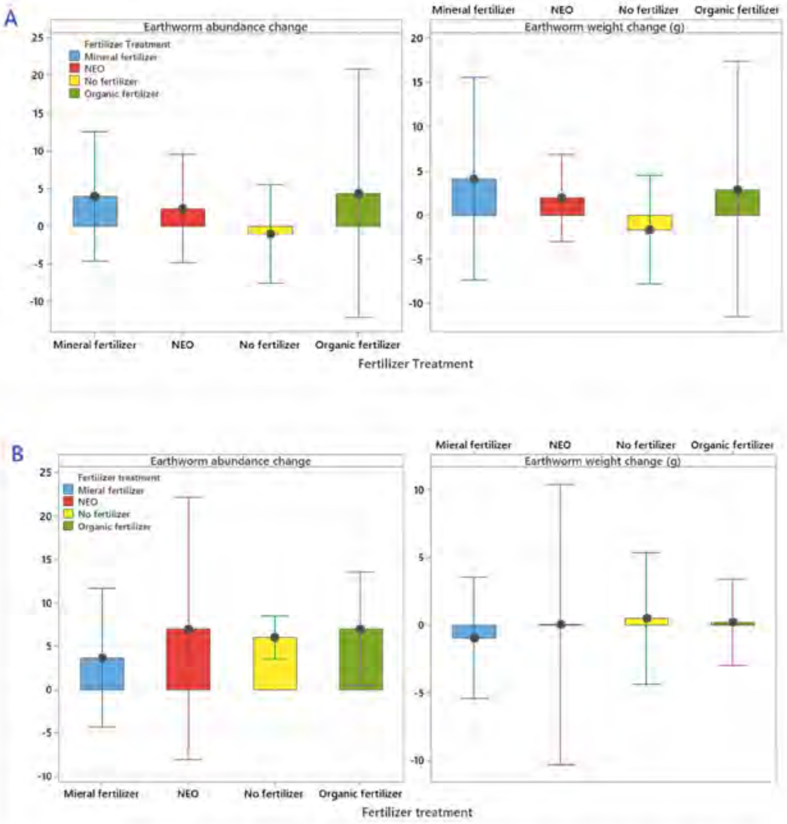


Figure 4. The immediate effect of different fertilization treatments on the abundance (left) and weight (right) of earthworms in (**A**) June 2021 and (**B**) June 2022. Error bars are individual standard deviations at a 95% confidence interval.

In the 2022 trial, however, the outcomes were slightly different. Although the abundance was increased for all fertilization treatments, the weights of living worms were almost identical to the beginning. The average abundance increment for organic fertilizer and NEO was 7, no fertilizer 6, and mineral fertilizer 3.67 earthworms (Figure 4B, Table S1). However, the average weight of all earthworms increased by 0.5 g for no fertilizer, 0.22 g for organic fertilizer, and 0.05 g for NEO. In comparison, mineral fertilizer reduced the total weight by 0.09 g (Figure 4B, Table S1). Nevertheless, changes in abundance or weight were insignificant between fertilizing treatments ($p = 0.69$ and $p = 0.83$, respectively).

Thus, the results indicated no adverse effects of fertilizing on the abundance and weight of earthworms, regardless of the fertilizer type used in both experiments.

4. Discussion

It is known that N fertilization affects the taxonomic composition of soil faunal communities, their population dynamics, and their feeding activity. However, it is not well understood if soil-dwelling organisms adapt to these external factors [59], especially when the external factor is a newly developed fertilizer (NEO). In this study, we investigated and compared the effects of different fertilization regimes on soil-dwelling organisms. We screened changes in soil faunal feeding activity under controlled conditions, the abundance of springtails, and the immediate effect of different fertilizers on the abundance and weight of earthworms under field conditions. The goal was to detect if NEO, a novel fertilizer with potentially toxic contents of nitrite, has any detrimental effects on soil-dwelling organisms compared to conventional fertilizers.

4.1. Soil Fauna Feeding Activity

The soil fauna feeding activity was evaluated at three intervals in pot experiments under controlled conditions; 0–7 weeks (early effect), 7–14 weeks (mid-term effect), and 14–21 weeks (late effect). Low (73 kg N ha^{-1}) and high (175 kg N ha^{-1}) rates of mineral fertilizer, NEO, organic fertilizer (untreated slurry), and a mixture of organic and mineral fertilizer were used as the fertilizing treatments.

Early effect analysis showed that low doses of fertilizer stimulated feeding activity irrespective of fertilizer type, while high amounts of fertilizer resulted in slightly less feeding activity. The only exception was the combination of organic and mineral fertilizer, which tended to have a higher feeding activity than no fertilizer. In line with our results, a grassland study showed that a high amount of organic fertilizer reduced soil fauna feeding activity within days after fertilizing [59]. Except for this, we could not detect any beneficial or detrimental early effect of fertilizers on soil fauna feeding activity. It may be argued that microbial biomass is promoted within the first weeks after high N fertilization resulting in alternate food sources for soil mesofauna, and they may have shifted away from the bait substrate [59], which explains lower feeding activity under higher fertilization.

The mid-term evaluation showed almost the same pattern as the evaluation of the early effect. The only difference was that at the higher N application rates, given organic and mineral fertilizer combination showed a slightly higher feeding activity than the organic fertilizer alone. Additionally, compared to no fertilizer, a high concentration of NEO showed a slightly increased soil faunal feeding activity. However, variations across fertilization treatments were smaller at the mid-term assessment than at the early effect evaluation, demonstrating that the initial stimulation gradually faded with time after fertilizing.

Finally, at the late effect evaluation, the initial stimulation by low amounts of fertilizer disappeared. Like during the early effect evaluation, higher amounts of fertilizer had lower feeding activities, and lower amounts of fertilizer had higher feeding activity irrespective of fertilizer type; whereas the difference among treatments was much smaller than in short- and mid-term evaluations, with less than five percent difference between the highest and lowest feeding activities.

Although similar to an earlier study [34], higher amounts of fertilizer, regardless of fertilizer type, initially showed a somewhat negative effect on soil faunal feeding activity, this detrimental effect progressively stabilized with time after fertilization. Furthermore, after some weeks of fluctuations in soil faunal feeding activity, a similar stabilizing effect has been reported in an oil palm plantation fertilized with different amounts of mineral N fertilizer [26]. The rationale for these transient effects might be the soil's buffering capacity and other soil chemical responses that gradually diminish fertilization's perturbation effect. Moreover, the soil fauna may be functionally redundant, conveying resilience to transient perturbations. Thus, we can summarize that neither NEO nor conventional fertilizers used in our experiment adversely affected the soil faunal feeding activity.

4.2. The Abundance of Springtails (Collembola)

In the grain field during summer, some weeks after fertilization, the numbers of springtails were almost identical among the plots fertilized with organic fertilizer and NEO. There were slightly fewer springtails than the latter two in the plots fertilized with mineral fertilizer. However, in the grass field, the plots fertilized with organic fertilizer supported an almost double and quadruple number of springtails compared to the plots fertilized with NEO and mineral fertilizer, respectively. Correspondingly, in the cereal field during fall, there were slightly more springtails in the plots fertilized with organic fertilizer and no fertilizer than in the plots fertilized with NEO and mineral fertilizer. The same pattern was observed in the grass field during the fall.

NEO had no adverse effects on the number of springtails after fertilization or after harvest; the number of springtails was generally lower during the summer than during the fall. It has been indicated that the abundance of springtails decreases after cattle slurry application [32]. However, this does not have been the case in our study. There might be two reasons for the apparent lack of response to organic fertilization. Springtails are moisture-dependent organisms [37]; therefore, it is a valid argument that sampling on a warm sunny Scandinavian day with limited moisture in the surface soil forced a major part of the springtail community to move deeper in the soil to avoid desiccation [60]. Moreover, more decaying plant matter as food for springtails may be available deeper in the soil after harvest [61].

Nonetheless, in line with our findings, another study showed almost no fertilization effect on the abundance of springtails [62], while another study indicated that fertilization increases the abundance of springtails [63]. Our study showed no adverse effect of NEO or other fertilizers on the abundance of springtails.

4.3. The Fate of Earthworms (Lumbricidae)

We used our developed method to investigate and compare the immediate effects of NEO and other fertilizers on earthworms. The experiment was repeated twice, once during summer 2021 and then in summer 2022. We targeted both the changes in the abundance and the average total weight of earthworms. The treatments were mineral fertilizer, NEO, organic fertilizer, and no fertilizer.

The results from the first experiment indicated no negative effect of fertilizer treatments on the abundance or weight of earthworms after eight days. Only the treatment with no fertilizer showed a slight reduction in the abundance and weight of earthworms. Moreover, roughly the same results were drawn from the second-year experiment. The only difference was that no fertilizer treatment did not lead to any reduction in the abundance or weight of earthworms.

In the case of NEO and organic fertilizer, a promoting effect of adding organic matter to the soil on the earthworm population was expected [18,30]. However, the concern was that excessive liquid slurry in a single dose might adversely affect earthworms [20]. Nonetheless, this did not occur with the amounts applied in our experiments. Moreover, mineral fertilizers might benefit earthworms through direct or indirect effects [19,20,22,64]. However, ammonia-based fertilizer potentially could have adverse effects on the earthworm population in the long run by lowering soil pH [18,65]; this was not the case in our experiments.

The concern might arise from the increasing number of earthworms in our experimental plots after eight days. Earthworms might have escaped their confinement even though we tested this before the study. Another possibility might be that tiny juveniles were contained in the soil before starting the experiment, which grew larger and became discoverable after eight days. Moreover, the most unlikely scenario might be that there were juveniles hatched from the cocoons within the experimental period. Notwithstanding, it is reasonable to argue that these error sources should have been identical for all experimental plots. Thus, it is logical to conclude that fertilization with NEO or any other fertilizer did not inhibit earthworms in the soil but supported an increase in number and activity.

5. Conclusions

NEO, the novel, plasma-treated nitrogen-enriched organic fertilizer, did not adversely affect soil faunal feeding activity, the abundance of springtails, and the abundance and weight of earthworms, as observed in pot and field trials. Moreover, fertilization with organic and mineral fertilizers was not seemed to harm the selected soil-dwelling organisms. Hence, NEO does not adversely affect the selected soil-dwelling organisms compared to conventional fertilization regimes commonly used in plant production today.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102314/s1; Table S1: Effects of different fertilizing treatments, including mineral fertilizer, NEO, organic fertilizer (untreated cattle slurry), organic fertilizer + mineral fertilizer (MF), and no fertilizer on soil fauna feeding activity (%) in the early effect, mid-term, and late effect evaluations, springtail abundance in summer and fall samplings at crop and grass fields, and the abundance and weight change (g) of earthworms, respectively. The Games–Howell pairwise comparison method at a 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.; Figure S1: Effects of all fertilizing treatments on soil fauna feeding activity (%) at seven weeks, 14 weeks, and 21 weeks after fertilizing. Individual standard deviations at a 95% confidence interval are used in the graphs.

Author Contributions: Conceptualization, H.M., T.C. and S.Ø.S.; methodology, T.C., H.M., S.Ø.S. and R.P.; software, H.M.; validation, H.M., T.C., S.Ø.S. and R.P.; formal analysis, H.M.; investigation, H.M. and R.P.; resources, T.C. and S.Ø.S.; data curation, H.M.; writing—original draft preparation, H.M.; writing—review and editing, H.M., S.Ø.S., P.D., T.C. and R.P.; visualization, H.M.; supervision, S.Ø.S. and T.C.; project administration, T.C.; funding acquisition, T.C. and S.Ø.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Research Council of Norway, grant number 309640 Plasmabehandlet husdyrgjødsel—gjødselvirkning, miljøpåvirkning og klimagassutslipp, and the APC was funded by Inland Norway University of Applied Sciences.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Data is accessible at https://osf.io/n4dyc/?view_only=25142c8ddd854758b8808d14fc792a76 (accessed on 3 August 2022).

Acknowledgments: The authors would like to acknowledge the administrative efforts of Morten Tofastrud and Elisabeth Røe throughout the project. Besides, we would like to thank the funders of the project, the Research Council of Norway and N2 Applied, along with those who provided us equipment and infrastructure required for our study: Norwegian Agricultural Extension service (NLR), Inland Norway University of Applied Sciences (INN), and Norwegian Centre for Organic Agriculture (NORSØK).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the result.

References

1. Mancus, P. Nitrogen Fertilizer Dependency and Its Contradictions: A Theoretical Exploration of Social-Ecological Metabolism*. *Rural Sociol.* **2007**, *72*, 269–288. [CrossRef]
2. Matson, P.A.; Parton, W.J.; Power, A.G.; Swift, M.J. Agricultural intensification and ecosystem properties. *Science* **1997**, *277*, 504–509. [CrossRef] [PubMed]
3. Lu, C.; Tian, H. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data* **2017**, *9*, 181–192. [CrossRef]
4. FAO. Fertilizers Indicators. Available online: https://www.fao.org/faostat/en/#data/EF (accessed on 18 July 2022).
5. Harris, S.L.; Thom, E.R.; Clark, D.A. Effect of high rates of nitrogen fertiliser on perennial ryegrass growth and morphology in grazed dairy pasture in northern New Zealand. *New Zealand J. Agric. Res.* **1996**, *39*, 159–169. [CrossRef]
6. Cinar, S.; Özkurt, M.; Cetin, R. Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (*Lolium multiflorum* L.) in central black sea climatic zone in turkey. *Appl. Ecol. Environ. Res.* **2020**, *18*, 417–432. [CrossRef]
7. Abraha, A.B.; Truter, W.F.; Annandale, J.G.; Fessehazion, M.K. Forage yield and quality response of annual ryegrass (*Lolium multiflorum*) to different water and nitrogen levels. *Afr. J. Range Forage Sci.* **2015**, *32*, 125–131. [CrossRef]
8. Savci, S. Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia* **2012**, *1*, 287–292. [CrossRef]
9. Siebert, J.; Sunnemann, M.; Auge, H.; Berger, S.; Cesarz, S.; Ciobanu, M.; Guerrero-Ramirez, N.R.; Eisenhauer, N. The effects of drought and nutrient addition on soil organisms vary across taxonomic groups, but are constant across seasons. *Sci. Rep.* **2019**, *9*, 639. [CrossRef]
10. Bünemann, E.K.; Schwenke, G.D.; Van Zwieten, L. Impact of agricultural inputs on soil organisms—A review. *Soil Res.* **2006**, *44*. [CrossRef]
11. European Commission. Sustainable Use of Nutrients: The Common Agricultural Policy Supports Farmers in the Safe and Efficient Use of Nutrients. Available online: https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability/low-input-farming/nutrients_en (accessed on 18 July 2022).
12. Bell, C.W.; Asao, S.; Calderon, F.; Wolk, B.; Wallenstein, M.D. Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biol. Biochem.* **2015**, *85*, 170–182. [CrossRef]
13. Yuan, J.; Zhao, M.; Li, R.; Huang, Q.; Rensing, C.; Shen, Q. Lipopeptides produced by B. amyloliquefaciens NJN-6 altered the soil fungal community and non-ribosomal peptides genes harboring microbial community. *Appl. Soil Ecol.* **2017**, *117–118*, 96–105. [CrossRef]
14. Körschens, M.; Albert, E.; Armbruster, M.; Barkusky, D.; Baumecker, M.; Behle-Schalk, L.; Bischoff, R. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: Results from 20 European long-term field experiments of the twenty-first century. *Arch. Agron. Soil Sci.* **2013**, *59*, 1017–1040. [CrossRef]
15. N2Applied. Nitrogen Enriched Organic Fertiliser. Available online: https://n2applied.com/ (accessed on 19 July 2022).
16. Graves, D.B.; Bakken, L.B.; Jensen, M.B.; Ingels, R. Plasma Activated Organic Fertilizer. *Plasma Chem. Plasma Processing* **2019**, *39*, 1–19. [CrossRef]
17. Ingels, R.; Graves, D.B. Improving the Efficiency of Organic Fertilizer and Nitrogen Use via Air Plasma and Distributed Renewable Energy. *Plasma Med.* **2015**, *5*, 257–270. [CrossRef]
18. Edwards, C.A.; Bohlen, P.J. *Biology and Ecology of Earthworms*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 1996; Volume 3.
19. Tiwari, S.C. Effects of organic manure and NPK fertilization on earthworm activity in an Oxisol. *Biol. Fertil. Soils* **1993**, *16*, 293–295. [CrossRef]
20. Edwards, C.A.; Lofty, J.R. Nitrogenous fertilizers and earthworm populations in agricultural soils. *Soil Biol. Biochem.* **1982**, *14*, 515–521. [CrossRef]
21. Edwards, C.A.; Lofty, J.R. *Biology of Earthworms*; Chapman and Hall; Wiley: London, UK; New York, NY, USA, 1977.
22. Hendrix, P.F.; Mueller, B.R.; Bruce, R.R.; Langdale, G.W.; Parmelee, R.W. Abundance and distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A. *Soil Biol. Biochem.* **1992**, *24*, 1357–1361. [CrossRef]
23. Guðleifsson, B. Impact of long term use of fertilizer on surface invertebrates in experimental plots in a permanent hayfield in Northern-Iceland. *Agric. Soc. Icel.* **2002**, *15*, 37–49.
24. McLaughlin, A.; Mineau, P. The impact of agricultural practices on biodiversity. *Agric. Ecosyst. Environ.* **1995**, *55*, 201–212. [CrossRef]
25. Geisseler, D.; Scow, K.M. Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biol. Biochem.* **2014**, *75*, 54–63. [CrossRef]
26. Wahyuningsih, R.; Marchand, L.; Pujianto, S.; Caliman, J. Impact of inorganic fertilizer to soil biological activity in an oil palm plantation. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *336*, 012017. [CrossRef]
27. Graenitz, J.; Bauer, R. The effect of fertilization and crop rotation on biological activity in a 90 year long-term experiment. *Bodenkultur* **2000**, *51*, 99–105.
28. Geisseler, D.; Lazicki, P.A.; Scow, K.M. Mineral nitrogen input decreases microbial biomass in soils under grasslands but not annual crops. *Appl. Soil Ecol.* **2016**, *106*, 1–10. [CrossRef]
29. Wei, M.; Hu, G.; Wang, H.; Bai, E.; Lou, Y.; Zhang, A.; Zhuge, Y. 35 years of manure and chemical fertilizer application alters soil microbial community composition in a Fluvo-aquic soil in Northern China. *Eur. J. Soil Biol.* **2017**, *82*, 27–34. [CrossRef]

30. Pommeresche, R.; Løes, A.-K. Relations between Agronomic Practice and Earthworms in Norwegian Arable Soils. *Dyn. Soil Dyn. Plant Glob. Sci. Books* **2009**, *3*, 129–142.
31. Pelosi, C.; Boros, G.; van Oort, F.; Schmidt, O. Soil Oligochaeta communities after 9 decades of continuous fertilization in a bare fallow experiment. *Soil Org.* **2020**, *92*, 129–141. [CrossRef]
32. Pommeresche, R.; Løes, A.-K.; Torp, T. Effects of animal manure application on springtails (Collembola) in perennial ley. *Appl. Soil Ecol.* **2017**, *110*, 137–145. [CrossRef]
33. Silva, D.M.d.; Jacques, R.J.S.; Silva, D.A.A.d.; Santana, N.A.; Vogelmann, E.; Eckhardt, D.P.; Antoniolli, Z.I. Effects of pig slurry application on the diversity and activity of soil biota in pasture areas. *Ciênc. Rural.* **2016**, *46*, 1756–1763. [CrossRef]
34. Mousavi, H.; Cottis, T.; Hoff, G.; Solberg, S.Ø. Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. *Sustainability* **2022**, *14*, 2005. [CrossRef]
35. Schinner, F.; Öhlinger, R.; Kandeler, E.; Margesin, R. *Methods in Soil Biolology*, 1st ed.; Springer: Berlin/Heidelberg, Germany, 1996. [CrossRef]
36. Fründ, H.-C.; Graefe, U.; Tischer, S. Earthworms as Bioindicators of Soil Quality. In *Biology of Earthworms*; Karaca, A., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 261–278.
37. Machado, J.; Oliveira Filho, L.C.; Santos, J.; Paulino, A.; Baretta, D. Morphological diversity of springtails (Hexapoda: Collembola) as soil quality bioindicators in land use systems. *Biota Neotrop.* **2019**, *19*, e20180618. [CrossRef]
38. Yara. YaraMila™. Available online: https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaramila/ (accessed on 20 July 2022).
39. Yara. YaraLiva™. Available online: https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaraliva/ (accessed on 20 July 2022).
40. Lumatek. ATS300W Specs Sheet. Available online: https://lumatek-lighting.com/lumatek-ats-300w/ (accessed on 21 July 2022).
41. Yiotis, C.; McElwain, J.C.; Osborne, B.A. Enhancing the productivity of ryegrass at elevated CO2 is dependent on tillering and leaf area development rather than leaf-level photosynthesis. *J. Exp. Bot.* **2020**, *72*, 1962–1977. [CrossRef] [PubMed]
42. TerraProtecta. The Bait-Lamina Test. Available online: http://www.terra-protecta.de/en/bait_strips.html (accessed on 24 July 2022).
43. Törne, E.v. Assessing feeding activities of soil-living animals. Bait-lamina-tests. *Pedobiologia* **1990**, *34*, 89–101.
44. Hamel, C.; Schellenberg, M.P.; Hanson, K.; Wang, H. Evaluation of the "bait-lamina test" to assess soil microfauna feeding activity in mixed grassland. *Appl. Soil Ecol.* **2007**, *36*, 199–204. [CrossRef]
45. Kratz, W. Bait-lamina test, general aspect, applications and perspectives. *Environ. Sci. Pollut. Res.* **1998**, *5*, 3.
46. Jänsch, S.; Scheffczyk, A.; Römbke, J. The bait-lamina earthworm test: A possible addition to the chronic earthworm toxicity test? *Euro-Mediterr. J. Environ. Integr.* **2017**, *2*, 5. [CrossRef]
47. ISO18311(E). Soil Quality–Method for Testing Effects of Soil Contaminants on The Feeding Activity Of Soil Dwelling Organisms in The Field–Bait-Lamina Test" for Approval for The Next Stage (FDIS) until 2015-04-17 (see Recommendation Paris-2). 2016. Available online: https://www.iso.org/standard/62102.html (accessed on 21 July 2022).
48. Gardi, C.; Montanarella, L.; Arrouays, D.; Bispo, A.; Lemanceau, P.; Jolivet, C.; Mulder, C.; Ranjard, L.; Römbke, J.; Rutgers, M.; et al. Soil biodiversity monitoring in Europe: Ongoing activities and challenges. *Eur. J. Soil Sci.* **2009**, *60*, 807–819. [CrossRef]
49. Simpson, J.E.; Slade, E.; Riutta, T.; Taylor, M.E. Factors Affecting Soil Fauna Feeding Activity in a Fragmented Lowland Temperate Deciduous Woodland. *PLoS ONE* **2012**, *7*, e29616. [CrossRef]
50. Rożen, A.; Sobczyk, Ł.; Liszka, K.; Weiner, J. Soil faunal activity as measured by the bait-lamina test in monocultures of 14 tree species in the Siemianice common-garden experiment, Poland. *Appl. Soil Ecol.* **2010**, *45*, 160–167. [CrossRef]
51. Verhoef, H.A.; Brussaard, L. Decomposition and nitrogen mineralization in natural and agroecosystems: The contribution of soil animals. *Biogeochemistry* **1990**, *11*, 175. [CrossRef]
52. Hopkin, S.P. *Biology of the Springtails (Insecta-Collembola)*; Oxford University Press: Oxford, UK, 1997; p. 330.
53. Tullgren. Ein sehr einfacher Ausleseapparat für terricole Tierformen. *Z. Angew. Entomol.* **1918**, *4*, 149–150. [CrossRef]
54. Berlese, A. *Apparecchio per Raccogliere Presto ed in Gran Numero Piccoli Arthropodi*; Kessinger Publishing, LLC: Whitefish, MT, USA, 1905; Volume 2, pp. 85–89.
55. O'Connor, F.B. The extraction of Enchytraeidae from soil. *Prog. Soil Zool.* **1962**, 279–285.
56. Bouché, M.B. Strategies lombriciennes. *Ecol. Bull.* **1977**, *25*, 122–132.
57. Graff, O. Unsere Regenwürmer (Lexikon für Freunde der Bodenbiologie). *Z. Pflanz. Bodenkd.* **1984**, *147*, 530. [CrossRef]
58. Sims, R.W.; Gerard, B.M.; London, L.S.; Association, E.B.W.S. *Earthworms: Keys and Notes for the Identification and Study of the Species; Linnean Society of London and the Estuarine and Brackish-Water Sciences Association*; Linnean Society of London and the Estuarine and Brackish-Water Sciences Association, Brill Archive: London, UK.
59. Birkhofer, K.; Baulechner, D.; Diekötter, T.; Zaitsev, A.; Wolters, V. Fertilization Rapidly Alters the Feeding Activity of Grassland Soil Mesofauna Independent of Management History. *Front. Ecol. Evol.* **2022**, *10*, 864470. [CrossRef]
60. Butterfield, J. Changes in decomposition rates and Collembola densities during the forestry cycle in conifer plantations. *J. Appl. Ecol.* **1999**, *36*, 92–100. [CrossRef]
61. Christiansen, K.A.; Bellinger, P.; Janssens, F. Chapter 55 - Collembola: (Springtails, Snow Fleas). In *Encyclopedia of Insects*, 2nd ed.; Resh, V.H., Cardé, R.T., Eds.; Academic Press: San Diego, CA, USA, 2009; pp. 206–210.
62. Gergócs, V.; Flórián, N.; Tóth, Z.; Szili-Kovács, T.; Mucsi, M.; Dombos, M. Crop species and year affect soil-dwelling Collembola and Acari more strongly than fertilisation regime in an arable field. *Appl. Soil Ecol.* **2022**, *173*, 104390. [CrossRef]

63. Wang, S.; Chen, H.Y.H.; Tan, Y.; Fan, H.; Ruan, H. Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. *Sci. Rep.* **2016**, *6*, 20816. [CrossRef]
64. Lalthanzara, H.; Ramanujam, S.N. Effect of fertilizer (NPK) on earthworm population in the Agroforestry system of Mizoram, India. *Sci. Vis.* **2010**, *10*, 159–167.
65. Ernst, D. *The Farmer's Earthworm Handbook: Managing Your Underground Money-Makers*; Lessiter Publications, Inc.: Brookfield, WI, USA, 1995.

Supporting Information:

Plasma treated nitrogen enriched manure does not impose adverse effects on soil fauna feeding activity or springtails and earthworms abundance

Table S1. Effects of different fertilizing treatments, including mineral fertilizer, NEO, organic fertilizer (untreated cattle slurry), organic fertilizer + mineral fertilizer (MF), and no fertilizer on soil fauna feeding activity (%) in the early effect, mid-term, and late effect evaluations, springtail abundance in summer and fall samplings at crop and grass fields, and the abundance and weight change (g) of earthworms, respectively. The Games-Howell pairwise comparison method at a 95% confidence interval is used to compare the differences between means. Means that do not share a letter are significantly different.

Soil fauna feeding activity (early effects, 7 weeks)	**Feeding activity (%)**	**Grouping**
Mineral fertilizer 73 kg N ha-1	78.13	A
Organic fertilizer 73 kg N ha-1	74.74	A
NEO type D 73 kg N ha-1	73.7	AB
Organic fertilizer + MF 175 kg N ha-1	61.98	ABC
No fertilizer	54.17	BC
NEO type D 175 kg N ha-1	49.22	C
Mineral fertilizer 175 kg N ha-1	46.35	C
Soil fauna feeding activity (mid-term effects, 14 weeks)	**Feeding activity (%)**	**Grouping**
Mineral fertilizer 73 kg N ha-1	58.44	A
NEO type D 73 kg N ha-1	55.99	A
Organic fertilizer + Mineral fertilizer 175 kg N ha-1	49.48	A
Organic fertilizer 73 kg N ha-1	48.96	A
NEO type D 175 kg N ha-1	45.57	A
No fertilizer	41.41	A
Mineral fertilizer 175 kg N ha-1	34.38	A
Soil fauna feeding activity (late effects, 21 weeks)	**Feeding activity (%)**	**Grouping**
NEO type D 73 kg N ha-1	49.74	A
Mineral fertilizer 73 kg N ha-1	49.48	A
Organic fertilizer 73 kg N ha-1	46.35	A
No fertilizer	46.09	A
NEO type D 175 kg N ha-1	45.83	A
Mineral fertilizer 175 kg N ha-1	45.57	A
Organic fertilizer + Mineral fertilizer 175 kg N ha-1	45.05	A
Soil fauna feeding activity all treatments (early, mid-term, and late effects averages)	**Feeding activity (%)**	**Grouping**
7 weeks	62.61	A
14 weeks	47.75	A
21 weeks	46.88	A
Springtail abundance summer sampling crop field	**Abundance per m²**	**Grouping**
Organic fertilizer	509.4	A
NEO	467	A
Mineral fertilizer	297.1	A
Springtail abundance summer sampling grass field	**Abundance per m²**	**Grouping**
Organic fertilizer	1528	A
NEO	807	A
Mineral fertilizer	467	A
Springtail abundance fall sampling crop field	**Abundance per m²**	**Grouping**
Organic fertilizer	212.3	A
No fertilizer	212.3	A

NEO	169.8	A
Mineral fertilizer	169.8	A
Springtail abundance fall sampling crop field	**Abundance per m²**	**Grouping**
Organic fertilizer	1486	A
No fertilizer	1486	A
NEO	1401	A
Mineral fertilizer	1316	A
Earthworm abundance change June 2021	**Abundance change**	**Grouping**
Organic fertilizer	4.33	A
Mineral fertilizer	4	A
NEO	2.33	A
No fertilizer	-1	A
Earthworm weight change June 2021	**Weight change (g)**	**Grouping**
Mineral fertilizer	4.07	A
Organic fertilizer	2.87	A
NEO	1.93	A
No fertilizer	-1.67	A
Earthworm abundance change June 2022	**Abundance change**	**Grouping**
Organic fertilizer	7	A
NEO	7	A
No fertilizer	6	A
Mineral fertilizer	3.67	A
Earthworm weight change June 2022	**Weight change (g)**	**Grouping**
No fertilizer	0.5	A
Organic fertilizer	0.22	A
NEO	0.05	A
Mineral fertilizer	- 0.95	A

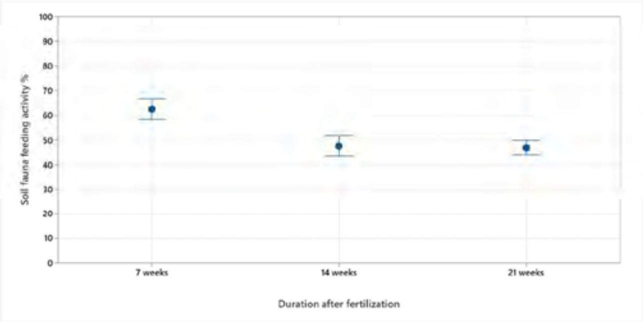


Figure S1. Effects of all fertilizing treatments on soil fauna feeding activity (%) at seven weeks, 14 weeks, and 21 weeks after fertilizing. Individual standard deviations at a 95% confidence interval are used in the graphs.

Nitrogen Enriched Organic fertilizer (NEO) elevates nitrification rates shortly after application but has no lasting effect on nitrification in agricultural soils

Hesam Mousavi (hesam.mousavi@inn.no)
Inland Norway University of Applied Sciences
Svein Øivind Solberg
Inland Norway University of Applied Sciences
Thomas Cottis
Inland Norway University of Applied Sciences
Peter Dörsch
Norwegian University of Life Sciences

Research Article

Keywords: Environment, innovative fertilizers, nitrogen, manure, sustainability, resilience

Posted Date: July 25th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2565156/v2

Abstract

In the face of population growth, rising food production costs, limited arable land availability, and farmland environmental degradation, novel technologies are crucial to bolster the resilience of global agri-food systems. Nitrogen-Enriched Organic fertilizer (NEO) is produced using a new method, where dinitrogen (N_2) is captured from the air through a plasma process and mixed with bio-based fertilizers as nitrate (NO_3^-) and nitrite (NO_2^-). This process leads to solid slurry acidification and a high NO_2^- content, potentially yielding toxic inorganic or organic N compounds. In this study, we investigated the impact of NEO, derived from cattle slurry and biogas digestate, on soil nitrification, which involves the conversion of NH_4^+ to NO_2^- and NO_3^- by aerobic autotrophic bacteria and archaea. We investigated and compared the potential nitrification rates in soil samples from two agricultural trials (cereal and grass) treated with NEO and other fertilizers after two consecutive fertilization years. Additionally, we examined the immediate nitrification response to NEO through 72-hour bottle incubations. Our results revealed that NEO significantly stimulated nitrification rates in agitated soil slurries, regardless of the feedstock used, surpassing rates observed in ammonium controls. Similarly, this pattern was also observed in loosely placed soil samples, with high nitrification rates occurring with NEO and ammonium chloride. Surprisingly, the differences in nitrification rates between field-fertilized soil samples were minimal and inconsequential, suggesting that while NEO exhibits a rapid boost in nitrification rates shortly after application, this effect is not sustained ≈ six months after fertilization under field conditions. Consequently, NEO indicates its potential as an environmentally benign fertilizer without adversely affecting soil nitrifier communities.

Introduction

The human population is increasing while arable land is becoming scarcer globally (Döös, 2002; Prăvălie et al., 2021). The United Nations (UN) predicts that the global population of almost 8 billion will increase to 9.8 bn in 2050 and more than 11 bn in 2100 (United Nations, 2017). At the same time, 12 million hectares of arable land are lost annually (United Nations, 2019), which means that productivity has to increase in the remaining area. This also means that agricultural systems become increasingly dependent on mineral nitrogen (N) fertilizers (Lu & Tian, 2017; Mancus, 2007; Matson et al., 1997). N is a critical nutrient for promoting plant growth; however, it is also one of the primary limiting nutrients in agroecosystems (Dong & Lin, 2020). Over the past six decades, agriculture productivity has significantly increased, tripling its output (FAO, 2017), while global N inputs into agriculture have remarkably grown, reaching an eight-fold increase (FAO, 2022).

Production of N fertilizers is highly energy-dependent, and thus energy price inflation is detrimental to energy security and endangers food production, specifically in low-income countries (Taghizadeh-Hesary et al., 2019). According to the U.S. Department of Agriculture (USDA), fertilizer prices have increased significantly in recent years due to several factors, including a limited supply of the required minerals, high energy costs, and a rise in global demand (USDA, 2022). This highlights the value of the circular economy and the importance of diversifying renewable and biobased resources in agroecosystems.

Although increased use of N fertilizers boosts crop productivity (Abraha et al., 2015; Cinar et al., 2020; Harris et al., 1996), it generates severe drawbacks (Upendra et al., 2019). Next to increasing fertilization expenses, more than 50% of the added N is lost as gaseous N (NO, N_2O, N_2, and NH_3) to the atmosphere and as nitrate (NO_3^-) to groundwater and waterways (Chen et al., 2020; Lassaletta et al., 2014; McAllister et al., 2012; Mulvaney et al., 2009). Today, there is twice as much mineral N in water, soil, and air systems globally than 100 years ago, owing primarily to the widespread use of mineral fertilizers (United Nations, 2020). Moreover, from a soil perspective, altering nutrients and causing imbalances with external additives lead to changes in functional microbial communities (Bell et al., 2015; Lu & Tian, 2017; Savci, 2012) as well as soil-dwelling organisms (Bünemann et al., 2006; Siebert et al., 2019), which may impose detrimental effects on soil biodiversity and the climate (European Commission, 2022).

Thus, urgent global actions are needed to alleviate the drawbacks of mineral N overuse. Among these actions are UNEP's "Halve Nitrogen Waste" campaign, which estimates global savings of $100 billion per year (considering half the value of global mineral fertilizer sales), and the European Green Deal, the European Commission's "Farm to Fork strategy" (European Commission, 2022; United Nations, 2020). The latter targets 20% less fertilizer consumption and a minimum 50% decrease in nutrient leaching by 2030. Aiming for these strategies and a drive towards a more sustainable future, the company N2 Applied (Asker, Norway) has developed a novel method to enrich organic slurry or biogas digestate with atmospherically derived nitrogen. Using this method, they create an N-rich, acidified, bio-based fertilizer, "Nitrogen Enriched Organic fertilizer (NEO)" (N2 Applied, 2022).

Atmospheric N is fixed as nitrogen oxides (NO_x) using either warm or cold plasma. This fixed Nis then brought into contact with the slurry or biogas digestate, forming nitrous acid (HNO_2) and nitric acid (HNO_3). This process lowers the pH of the mixture and impacts microbial activity (N2 Applied, 2022). Furthermore, slurries treated with NEO are filtered and transformed into a highly liquefied state, which sets them apart from conventional animal manures. This enhanced liquefaction facilitates a more precise application of NEO-treated slurries to the field. (Graves et al., 2019; Ingels & Graves, 2015; N2 Applied, 2022). The N2 Applied device's compact size also enables local NEO production for farmers, boosting the self-sufficiency of stakeholders (N2 Applied, 2022).

NEO is a new product, and possible adverse effects on soil-dwelling organisms and communities and soil nutrient cycling should be evaluated before being introduced to global markets. Hence, using different approaches, this study investigates the impact of NEO on potential nitrification, a crucial microbially mediated function in the soil N cycle (Creamer et al., 2022; Prosser & Nicol, 2012; Schinner et al., 1996; Zwetsloot et al., 2022), and compares it to conventional fertilizers used in agriculture today.

Nitrification activity is a putative biological indicator of soil quality, biodiversity, and multifunctionality (Bünemann et al., 2018; Griffiths et al., 2016; Zwetsloot et al., 2022), as it is a critical stage in the soil N cycle (Nelson et al., 2016) and has been shown to be sensitive to perturbations (Stein, 2019). Nitrification, the oxidation of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-), is mediated by ammonia-oxidizing bacteria (AOB) and archaea (AOA) as well as anaerobic ammonium oxidizers (anammox) (Kartal et al., 2012; Killham, 1998; Purkhold et al., 2000; Ward, 2008). Environmental conditions, including salinity, temperature, oxygen availability, and pH, determine the nitrification rate in natural systems (Ward, 2008).

Nitrification is particularly influential in agricultural systems by regulating the accessibility of N from fertilizers for plants (Ward, 2008). Nitrate is more mobile than other mineral N species across the soil matrix, significantly affecting N retention in the system (Norton & Ouyang, 2019). N fertilization elevates the nitrification rate, as documented in field experiments (Wang et al., 2019), incubation experiments, and analyses of the genes involved in the nitrification process (Bi et al., 2017; Ouyang et al., 2018). However, as nitrification increases N accessibility for the plants (C.F. Drury, 2007), nitrate leaching and gaseous nitrous oxide (N_2O) formation also increase, decreasing the N retention and plant availability of N (Beeckman et al., 2018; Fowler et al., 2013). Hence, the reduction of nitrification becomes desirable in situations where there is a potential risk of N losses and environmental pollution, as well as a decrease in the efficiency of N fertilizers (Tilman et al., 2002).

In earlier investigations, we concluded that NEO slurry has no adverse effect on soil fauna feeding activity, an essential factor in soil nutrient cycling (Mousavi, Cottis, Hoff, et al., 2022; Mousavi, Cottis, Pommeresche, et al., 2022), or the abundance of springtails and earthworms (soil faunal communities) (Mousavi, Cottis, Pommeresche, et al., 2022). In this study, we assessed the nitrification potential in two field trials, one grass and the other cereals, where NEO slurry was applied to the soil for two consecutive years in the same plots. The NEO slurry was compared to conventional mineral and organic fertilizers commonly used at standard application rates. We hypothesized that applying NEO slurry would not negatively impact the potential activity of soil nitrifier communities under field conditions. Additionally, we investigated the immediate short-term effects of NEO slurry and NEO biogas digestate on soil nitrification in laboratory settings. Here, we hypothesized that the acidity of NEO would temporarily slow down the nitrification process.

Materials And Methods

Experimental design

We used three experimental setups to determine the impact of NEO on nitrification potential: 1) field-fertilized soil, 2) lab-fertilized soil in stirred soil slurries, and 3) lab-fertilized soil incubated as loose soil.

Field-fertilized soil

The first setup consisted of a field trial conducted at two distinct locations with varying fertilization regimes. The trial's initial location involved the cultivation of cereals and was situated at the experimental farm of Inland Norway University of Applied Sciences, Blæstad (60°49'11.7" N 11°10'48.4" E). Meanwhile, the second location focused on a perennial grass meadow and was situated at an experimental farm in Stjørdal, Trøndelag (63°20'33.4" N 10°17'56.9" E).

At the first location, the spring wheat (*Triticum aestivum* L.) variety "Mirakel" (220 kg ha^{-1}) was planted in 2020, while the barley (*Hordeum vulgare* L.) variety "Rødhette" (180 kg ha^{-1}) was planted in 2021. At the second location, the field was planted one year before our experiment started with a perennial grass crop mixture of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.), and red clover (*Trifolium pratense* L.). In cereals, the herbicides Ariane S (Corteva Agriscience, Puerto Rico) and Roundup (Bayer, Germany) were sprayed: Ariane S in June and Roundup at the end of the growing season. In the grass meadow, no herbicides were applied.

To minimize any potential marginal effects, we standardized the size of all fertilizer plots to 10 × 3 m in the cereal field, with a harvested area of 1.5 × 8.5 m within each plot, and 8 × 2.5 m in the grass field, with a harvested area of 6.5 × 1.5 m.

In the cereal field, fertilization was conducted once before planting each year, specifically on April 22, 2020, and April 27, 2021. In contrast, the grass plots received fertilizer application twice yearly – first in early spring (April 27, 2020, and May 4, 2021) and then after the first harvest (June 24, 2020, and June 15, 2021). The experimental setup utilized a standard randomized complete block design (RCBD) with four replicates to ensure rigorous and reliable data collection.

Background information on the soil was obtained from analysis performed at Eurofins soil laboratory (https://www.eurofins.no/agro-testing/soil). The soil texture in the cereal field was classified as a sandy clay loam with a pH of 7.4 and 4.5 percent organic matter. The phosphorus status was normal (11 mg available P per 100 g dry soil), and the potassium status was below average (5 mg available K per 100 g dry soil). The total pore volume was 41.4%, and the field water capacity was 33.6% VWC. The soil texture in the grass field was classified as a clay loam consisting of around 10 percent clay, with a pH of 5.7 and 5.1 percent organic matter. The phosphorus and potassium statuses were average (8 mg available P per 100 g dry soil and 7 mg available K per 100 g dry soil, respectively), but the potassium reserve was high (140 mg K-HNO_3 per 100 g dry soil).

In both field experiments, the treatment plots received the following fertilizers consistently over two consecutive years before we collected soil samples for nitrification measurements: mineral fertilizer (Yara Mila 18-3-15: Nitrate 8.3%; ammonium. 9.3%; Yara, Oslo Norway) (Yara, 2021), NEO cattle slurry (hereafter NEO) (N2 Applied, Asker, Norway) (N2 Applied, 2022), organic fertilizer (the same untreated cattle slurry used to produce NEO), and no fertilizer (control).

NEO had a total N content of 3407 mg L^{-1} consisting of 1480 mg L^{-1} NH_4^+-N, 777 mg L^{-1} NO_2^--N, and 1150 mg L^{-1} NO_3^--N and a pH of 5.3. By contrast, cattle slurry (pH 7.3) had a total N content of 1953 mg L^{-1}, consisting of 1804 mg L^{-1} NH_4^+-N and 149 mg L^{-1} NO_3^--N.

The cereal field received the following fertilization treatments: (1) no fertilizer, (2) organic fertilizer 41 tons ha^{-1}, (3) NEO 37.6 tons ha^{-1}, and (4) mineral fertilizer 666.6 kg ha^{-1}. The grass field received the following fertilizer amounts: (1) no fertilizer, (2) organic fertilizer 41 tons ha^{-1} + 30.5 tons ha^{-1}, (3) NEO 37.5 tons ha^{-1} + 28 tons ha^{-1}, and (4) mineral fertilizer 650 kg ha^{-1} + 500 kg ha^{-1}.

The NEO and mineral fertilizer amounts were carefully adjusted to contain equivalent mineral N per hectare. Specifically, NEO and mineral fertilizer were applied to provide 120 kg N per hectare in the cereal plots. In the grass plots, the application rates were adjusted to achieve 210 kg N per hectare.

Mineral fertilizer was administered in pellet form, whereas organic fertilizer and NEO were in liquid form. To ensure uniform distribution of particles in the liquid, all fertilizer stocks were thoroughly swirled before bottling. Fertilizers were manually scattered onto the soil surface using containers and promptly harrowed into the soil with a tractor before planting or dispersed on the grass surface during the growing season. It is important to note that the fields did not receive any irrigation.

Regarding the cereal trial, the weather data for 2020 showed that it was 1.4 °C cooler than the average temperature and received less than half of the usual precipitation. In contrast, the weather in 2021 had a normal average temperature, but there was about 20% more precipitation than usual. Consequently, the cereal yield in 2021 was above average, likely influenced by the favorable precipitation conditions.

For the grass trial, the weather data indicated no extreme climatic events, such as unusual temperatures or precipitation, during the two growing seasons. The grass yields were average in both years. While specific weather data is not provided in this context, it is essential to emphasize that the typical weather conditions experienced should not introduce any bias to our study results. However, if necessary, specific climate conditions can be obtained from the Norwegian meteorological online database (yr.no, 2023).

On October 20, 2021, soil sampling was conducted at both field locations. The sampling day saw temperatures around 5-6 °C, following rainy days at both sites. To ensure representative samples, ten diametric cores were collected from each experimental plot, reaching a depth of 20 cm. The cores, with a diameter of 80 mm, were carefully composited to form a bulk sample, totaling approximately 1 kg of field-moist soil. During the process, coarse rocks and roots were removed from the composite sample.

Each bulk sample was transferred to a plastic zipper bag to preserve the soil moisture and prevent desiccation. Following established protocols (Schinner et al., 1996), these bags were stored in a cooling room at 4°C until further processing in the laboratory, as per the procedures (Öhlinger et al., 1993) described. This approach ensured that the soil samples were adequately preserved and maintained at appropriate conditions for subsequent laboratory analysis.

To obtain homogenous soil samples for the laboratory analysis, we sieved the soil to 3 mm and removed all remaining plant debris. Since the soil moisture was still higher than required for lab analysis, the sieved soil was kept in open zipper bags (gas exchange) at room temperature for 24 hours before transferring back to the refrigerator. The soils were always kept refrigerated pending laboratory experiments.

Sieving soil elevates soil biological activity by destroying soil aggregates, exposing soil fractions to oxygen, and making increased amounts of nutrients or substrates accessible. Therefore, the soils were stored for six days before experimentation to recover the original biological activity in the soil (Öhlinger et al., 1993).

Gravimetric soil moisture was evaluated according to the protocol by drying (Kellogg Biological, 2019). The gravimetric water content in the cereal field soil was estimated to be 24.9%, and in the grass field soil, 33.9% on average.

Next, we measured and weighed 12.5 g of cereal field soil and 13.5 g of grass field soil, equivalent to approximately 10 g of soil dry weight, accounting for gravimetric water content. These samples were taken from various fertilization treatments and placed into 120 ml serum bottles. We added 50 ml of deionized (DI) H_2O to each bottle, ensuring consistent conditions across all samples.

Subsequently, all the bottles were promptly capped using rubber septa and aluminum crimp seals. To create a uniform environment for incubation, we placed the capped bottles into a horizontal shaker and incubated them for 66 hours at room temperature.

Lab-fertilized soil incubated as agitated soil slurry

We were interested in instantaneous nitrification responses to the different fertilizers in a second set of experiments. For this, we combined soil from the different treatment plots at each field into two bulk samples, one for the cereal and one for the grass field. This was done to rule out confounding effects of long-term field treatment. Then, 12.5 g of cereal field soil and 13.5 g of grass field soil were weighed into 36 × 120 ml serum bottles (18 each).

Next, six fertilization treatments with three replicates were prepared; untreated cattle slurry (Raw S), untreated biogas digestate (Raw D), NEO made from cattle slurry (NEO S), NEO made from biogas digestate (NEO D), untreated cattle slurry acidified with HCl (Raw S acidified), and as a positive control a concentrated NH_4Cl solution. The fertilizer amounts were adjusted based on their NH_4^+ content (the dominant N form) to translate to 170 kg NH_4^+-N ha^{-1}, based on 1.2 g cm^{-3} soil bulk density and 5 cm soil depth, yielding 17 g fertilizer m^{-2}, or 0.283 mg N g^{-1} dry weight soil, or 2.83 mg NH_4^+-N $bottle^{-1}$.

Raw S (pH 7.6) contained 1606 mg NH_4^+-N L^{-1}, raw D (pH 8.1) 3020 mg NH_4^+-N L^{-1}, NEO S (pH 5.2) 1732 mg NH_4^+-N L^{-1}, NEO D (pH 5.1) 2580 mg NH_4^+-N L^{-1} and acidified raw S (pH 5.1) 1606 mg NH_4^+-N L^{-1}. Thus, 1.76 ml raw S, 0.94 ml raw D, 1.64 ml NEO S, 1.10 ml NEO D, and 1.76 ml acidified raw S were applied to designated bottles and filled with 50 ml DI H_2O. The ammonium chloride treatments (pH 6.8) were prepared by mixing the soils with 50 ml of a solution containing 216.5 mg N L^{-1}.

All the bottles were capped instantly using rubber septa and aluminum crimp seals and set into a horizontal shaker, where they were incubated for 43 h at room temperature.

Lab-fertilized soil incubated as loose, non-agitated soil

To explore the effect of soil disintegration and shaking on nitrification activity, lab-fertilized soils were incubated loosely for 73 hours. Since the effect of fertilization in the slurried soils was similar for cereal and grass soils, this experiment was only conducted with soil from the cereal field, which had a higher pH and a better-buffering capacity than the soil from the grass field.

First, three sets of 15 × 50 ml sterile centrifuge tubes filled with 12.5 g of cereal field soil (i.e., corresponding to approximately 10 g dry-weight soil) were arranged. Then, five fertilization treatments with three replicates were applied to all three sets simultaneously: raw S, raw D, NEO S, NEO D, and ammonium chloride.

The fertilizer amounts were adjusted to correspond to 85 kg NH_4^+-N ha^{-1} based on a bulk density of 1.2 g cm-3 and a soil depth of 5 cm. This yielded 8.5 g fertilizer m^{-2}, or 0.142 mg N g^{-1} soil, and therefore 1.42 mg NH_4^+-N $tube^{-1}$. Thus, 0.88 ml raw S, 0.47 ml raw D, 0.82 ml NEO S, and 0.55 ml NEO D were applied to designated tubes. In addition, an ammonium chloride solution was prepared by mixing 0.54 g NH_4Cl in 100 ml DI H_2O, and 1 ml of the solution was applied to designated tubes. The tubes were capped and incubated for 73 h at room temperature.

Determining nitrification rates

Nitrification rates were determined as the NO_3^- + NO_2^- accumulation rate in the three different experiments using colorimetric assays and a spectrophotometer (Infinite® F50, TECAN Life Sciences, Männedorf, Switzerland).

The nitrite concentration (NO_2^-) was measured according to the Greiss reaction assay (Griess, 1858; Keeney & Nelson, 1983; Killham, 1990; Thion & Prosser, 2014; Wang, 2010). Nitrite + nitrate (NO_2^- + NO_3^-) concentration was determined following the assay adapted from Doane & Horwáth (2011) (Doane & Horwáth, 2011), using Vanadium (III) chloride (VCl_3), which oxidizes nitrite to nitrate.

Potential nitrification rates of field-fertilized soil

Nitrification rates in the field fertilized soils were estimated from the NO_2^- + NO_3^- increase in the soil slurries throughout incubation in a shaker. The bottles were removed from the shaker to subsample the slurry, followed by 10 minutes of standing still on the bench for the soil to settle. When the top layer appeared clear, 1 ml of the liquid was extracted using a syringe and transferred to 2 ml Eppendorf tubes. Afterward, the bottles were returned to the shaker. Constant shaking aerates the soil and relieves diffusional constraints (C.F. Drury, 2007). The bottles were sampled four times at 0, 19, 44.5, and 66 h into the incubation. The extracted subsamples were centrifuged at 10000 rpm and 4°C for 10 minutes Before determining NO_2^- and NO_2^- + NO_3^- concentrations as described above.

Considering nitrification is pH-sensitive, and the rate declines at low pH values (Ste-Marie & Paré, 1999; Zebarth et al., 2015), the soil pH was screened before and after the experiment using a handheld pH meter (Hach- H-Series H160, Loveland, CO, USA) equipped with an ISFET sensor (Mettler Toledo, Stockholm, Sweden).

Lab-fertilized soil incubated as soil slurries

All procedures, including incubation, extraction, and determination of NO_2^- + NO_3^-content in the extracts, were like the procedures for field-fertilized soil samples (see section 2.2.1), with two exceptions. First, three extraction rounds occurred at 0, 19, and 43 hours after incubation. Second, due to the high nitrate contents coming along with the NEO fertilizers, the extracts had to be diluted ten times with DI H_2O after the first extraction round and 20 times after the second and third extraction. Like in the experiment with field-fertilized soil, all samples were screened for soil pH change before and after incubation.

Lab-fertilized soil loosely placed

Accumulation of NO_2^- + NO_3^- over time was measured by sacrificing each three tubes per treatment 1, 25, and 73 h into the incubation. NO_2^- + NO_3^- was extracted by adding 30 ml 2 M KCL and shaking for one hour. After that, the tubes were left still for 10 minutes to settle before using 1 ml of the supernatant for NO_2^- + NO_3^- analysis, following the same procedure described in section 2.2. and 2.2.1.

Data handling and statistical analyzes

The spectrophotometry data regarding NO_2^- + NO_3^- accumulation was first registered and sorted in MS Excel (MS Office 365, Redmond, WA, USA). Then, the nitrite (NO_2^-) and nitrate (NO_3^-) accumulation rates were computed and plotted against incubation time using polynomial regression. Since the nitrite accumulation was negligible, nitrification potentials were calculated from (NO_2^- + NO_3^-)-N accumulation rate and expressed as µg N g dry weight $soil^{-1}$ day^{-1}. An ANOVA test and general linear model (GLM) were used in Minitab 21 (Minitab LLC, State College, PA, USA) to assess the difference in potential nitrification rates vs. fertilization treatments and replicates. In addition, Tukey pairwise comparison at a 95% confidence interval was used to group and plot the data. Error bars in the plots were calculated using individual standard deviations.

Results

Field-fertilized soil

Nitrification potentials in soil samples that had undergone different fertilization treatments in the field indicated no significant impact of fertilization in either cereal or grassland soils ($p = 0.98$ and $p = 0.68$ in the cereal and grass fields, respectively).

For the cereal field, the control treatment (no fertilizer) had a slightly higher but non-significant ($NO_2^- + NO_3^-$)-N accumulation rate (32.8 µg g dry weight soil^{-1} day^{-1}) than soils that had received mineral fertilizer (31.4 µg g DW soil^{-1} day^{-1}), organic fertilizer (31.3 µg g DW soil^{-1} day^{-1}), and NEO (31.1 µg g DW soil^{-1} day^{-1})(Figure 1A, Figure S1A-D to S4A-D, Table S1). in the grassland experiment, organically fertilized soil had a slightly higher nitrification rate (19.2 µg g DW soil^{-1} day^{-1}) than soil which had received mineral fertilizer (18.1 µg g DW soil^{-1} day^{-1}), no fertilizer (17.4 µg g DW soil^{-1} day^{-1}), or NEO (15.9 µg g DW soil^{-1} day^{-1}) (Figure 1B, Figures S5A-D to S8A-D, Table S1). However, as mentioned, the differences were negligible and insignificant. Moreover, the nitrification rate was generally lower in the grass field than in the cereal field (Figures 1A-B, Table S1).

The native pH in cereal and grass field soils was 7.4 and 5.7, respectively. At the start of the incubation, the average pH of fertilized cereal soils (n=4) varied between ca. 6.7-7.1. The native pH of fertilized grass field soils (n=4) varied between ca. 5.4-5.6, i.e., no fertilizer, 5.4; organic fertilizer, 5.6; mineral fertilizer, 5.4; and NEO, 5.5. As expected, adding ammonium in the form of different fertilizers decreased the pH during the 66-hour incubation, i.e., cereal field samples: no fertilizer, 6.4; organic fertilizer, 6.4; mineral fertilizer, 6.5; and NEO, 6.5, and grass field samples: no fertilizer, 4.6; organic fertilizer, 4.8; mineral fertilizer, 4.6; and NEO, 4.6. the pH of the grass field samples decreased more than that of the cereal field samples (Figures 2A-B).

Lab-fertilized soil incubated as agitated soil slurries

Measuring nitrification potentials in agitated soil slurries directly after amending with different fertilizers revealed significant differences in nitrification rates between fertilization treatments in cereal and grass field soils ($p \leq 0.001$ and $p \leq 0.001$, respectively).

In both soils, NEO made from biogas digestate (NEO D) had the highest nitrification rates with average values of ~250 µg N g DW soil^{-1} day^{-1}, followed by NEO S indicating a higher ($NO_2^- + NO_3^-$)-N accumulation rate (257.3 and 108.4 µg g DW soil^{-1} day^{-1}, respectively) than ammonium chloride (54 µg g DW soil^{-1} day^{-1}), untreated biogas digestate (Raw D)(46.5 µg g DW soil^{-1} day^{-1}), acidified untreated slurry (Raw S acidified) (41.9 µg g DW soil^{-1} day^{-1}), and untreated slurry (Raw S) (34.9 µg g DW soil^{-1} day^{-1})(Figure 3A, Figures S9A-D to S14A-D, Table S1). Similarly, within treatments in grass field soil, NEO D and NEO S indicated a higher nitrification rate (253.7 and 123.7 µg g DW soil^{-1} day^{-1}, respectively) than ammonium chloride (28.7 µg g DW soil^{-1} day^{-1}), raw D (20.3 µg g DW soil^{-1} day^{-1}), raw S (17.7 µg g DW soil^{-1} day^{-1}), and raw S acidified (12.1 µg g DW soil^{-1} day^{-1})(Figure 3B, Figures S15A-D to S20A-D, Table S1). Except for NEO D and NEO S, which indicated identical nitrification rates in cereal and grass field soils, similarly to the first experiment, nitrification rates were lower in the grass field soil than in the cereal field soil.

The pH changed less throughout the 43-hour incubation as compared with the previous experiment, which used 66 h of incubation. The initial average pH of fertilized cereal samples (n=3) varied between ca. 6.7-7.5 (i.e., raw S, 7.4; raw D, 7.4; NEO S, 6.9; and NEO D, 6.9; raw S acidified 6.7; and ammonium nitrate, 7.1). Additionally, the native average pH of fertilized grass field soils (n=3) varied between ca. 5.2-6.7 (i.e., raw S, 6.7; raw D, 6.6; NEO S, 5.4; and NEO D, 5.5; raw S acidified 5.2; and ammonium nitrate 5.4) (Figures 4A-B). However, within the cereal field samples, after the incubation period, only raw S and raw D exhibited a slight pH reduction (6.9 and 7.1, respectively), whereas other treatments had similar pH to the beginning (Figure 4A). Nonetheless, within grass field samples, NEO S, NEO D, raw S acidified, and ammonium chloride exhibited a slight increment in average pH (n=3) (NEO S, 6.1; NEO D, 6.5; raw S acidified, 5.1; and ammonium chloride, 5.6) whereas raw S and raw D had relatively the same pH as the start (Figure 4B).

Lab-fertilized soil loosely placed

When incubating freshly amended soil without agitation, there were significant differences in $NO_2^- + NO_3^-$ accumulation between fertilization treatments ($p \leq 0.001$).

Like with the slurried soil, NEO D showed the largest nitrification rate (60.1 µg g DW soil^{-1} day^{-1}), which was significantly larger than that of ammonium chloride (24.5 µg g DW soil^{-1} day^{-1}), and NEO S (20.5 µg g DW soil^{-1} day^{-1}). However, raw D (12.9 µg g DW soil^{-1} day^{-1}) and Raw S (0 µg g DW soil^{-1} day^{-1}) had the lowest average nitrification rate (Figure 5, Figures S21A-D to S25A-D, Table S1).

Discussion

The production of Nitrogen-Enriched Organic fertilizers (NEO) represents a novel technology that necessitates comprehensive evaluation under field and laboratory conditions to assess potential adverse effects on soil biota before scaling up and commercialization. While NEO is bio-based, its high nitrite content and low pH raise concerns regarding forming harmful radicals and toxic compounds in the soil.

Soil biota play a pivotal role in soil functions, and in this study, we focused on one of the critical aspects of soil nutrient cycling – nitrification. By assessing changes in potential nitrification (Hu et al., 2011; Robertson, 2015), we aimed to evaluate whether NEO affects or hampers the activity of nitrifying soil microbes compared to commonly used mineral and organic fertilizers in agriculture.

Understanding the potential impact of NEO on nitrification is vital as it can provide crucial insights into the sustainability and safety of utilizing this fertilizer technology. Through our research, we sought to contribute to the knowledge base required for making informed decisions about NEO's applicability in agricultural practices, ensuring responsible and environmentally-friendly fertilization approaches.

Minimal long-term fertilization effects in the field

In our assessment of nitrification rates in field-fertilized soil samples taken during autumn, approximately 5-6 months after fertilization, we found no significant difference in nitrification potentials among the various fertilization treatments in the cereal and grass fields. Notably, the extended period between fertilization and sample collection allowed the removal of added N from the soil through plant uptake, leaching, or emission and the subsequent recuperation from potentially harmful effects. Hence, we may conclude that NEO, produced from cattle slurry or biogas digestate and applied in these trials, does not exhibit a legacy effect on soil nitrification during autumn, even after two consecutive years of application.

However, our initial expectation of observing distinct potential nitrification rates between fertilized and unfertilized soil, as indicated by similar studies (Mohanty et al., 2022; Raglin et al., 2022; Wang et al., 2018), was not observed in our experiments. This discrepancy could be attributed to the relatively short two-year treatment period or the possibility that nitrification potentials are sustained through nitrogen mining from the relatively high soil organic matter (SOM) content in the non-fertilized treatment. Additional treatment years would be necessary to draw definitive conclusions regarding the legacy effects of fertilization, including NEO.

Furthermore, the type and mode of fertilization also impact the taxonomic composition of bacterial and archaeal communities and their interactions at various taxonomic levels. The composition of the nitrifier community is influenced, among other factors, by spatial variability in ammonia availability (Rütting et al., 2021), which, in turn, depends on clay content. In addition to fixed ammonium, clay minerals can sorb complex N-containing compounds, which can subsequently be mineralized to NH_4^+ upon release (Nieder et al., 2011). While we did not specifically study the taxonomic composition of the underlying nitrifier communities, other studies have reported on functional redundancy among different taxonomic groups of nitrifiers as a potential reason for the lack of a fertilizer-specific response in field soils (Gu et al., 2017; Raglin et al., 2022; Wu et al., 2011).

Nitrification rates were generally lower in the grass field than in the cereal field soil. This was expected since nitrification is a pH-dependent process (DeForest & Otuya, 2020), and the grassland soil had a markedly lower pH than the cereal field soil.

Major short-term fertilization effect in lab-fertilized soils

Interestingly, fertilizer-amended cereal and grass field soils incubated as agitated soil slurries did not show any difference; despite the lower pH, NEO made from biogas digestate (NEO D), and NEO made from cattle slurry (NEO S) stimulated the nitrification rates more than other fertilization treatments, even though identical amounts of NH_4^+ were applied. The loosely placed lab-fertilized soil samples showed much of the same pattern, albeit with lower nitrification rates than the agitated soil slurries. Again, NEO D stimulated nitrification rates more than the other fertilization treatments. The difference to agitated soil slurries was that NEO S and ammonium chloride had almost identical nitrification rates. This confirms the hypothesis that ammonium accessibility is not solely responsible for active nitrifier communities (Raglin et al., 2022).

Since NEO fertilizers have considerably higher NO_2^- and NO_3^- contents and lower pH values than untreated slurries, one would expect lower nitrification rates in NEO slurries, for instance, because of product inhibition. We found the opposite, suggesting that NEO stimulated nitrification transiently irrespective of its NH_4^+ content through some other mechanism. The organo-chemical composition of NEO is unknown, but it is conceivable that the radicals formed by the plasma process form volatile organic compounds (VOCs) that have been shown to stimulate nitrification in one study (Mohanty et al., 2019). The fact that we found the same stimulation pattern across treatment in the two different soils indicates a NEO-related factor independent of soil. Another reason for the stimulation by NEO could be that NEO's C/N ratio is smaller than that of untreated cattle slurry or biogas digestate due to the N enrichment. The lower C/N ratio of NEO might have reduced the immobilization of NH_4^+ and thus sustained a higher nitrification rate (Watson et al., 2002).

Concerning NEO's high acidity, we expected that the acidity of NEO would temporarily slow down nitrification transiently. However, in our lab experiments, nitrification rates increased with the addition of $NH_4^{+,}$ and this effect was surprisingly more pronounced with NEO D and NEO S than in the other amendments. By contrast, nitrification potentials in NEO field treatments were indistinguishable from those of other fertilizer treatments after two years of treatment, evidencing that NEO's low pH or high nitrite content does not affect the activity of soil nitrifiers throughout the course of a year.

Thus, considering the potential benefits of NEO as a bio-based fertilizer, this study confirms that NEO could be potentially harmless to use in agroecosystems. However, the study sheds light on the short-term effects of NEO and other fertilizers on nitrification in two sub-boreal soils, and it does not indicate which physiochemical drivers or changes in the soil microbial communities occur. Therefore, future studies into mechanisms regulating nitrification rates under different fertilization treatments should include data on nitrifier communities' taxonomic composition.

Conclusions

Field and laboratory experiments were conducted to investigate and compare the impact of Nitrogen Enriched Organic fertilizers (NEO) on potential nitrification rates. The main objective was to assess whether newly developed bio-based fertilizers, NEO produced from cattle slurry and biogas digestate, influence the activity of soil-nitrifying communities. Despite NEO fertilizers' high nitrite content and low pH, our laboratory experiments demonstrated that NEO did not inhibit nitrification; instead, it directly stimulated nitrification shortly after application. This stimulating effect was observed in agitated soil slurries and loosely-placed soil samples. However, when we examined the field soil samples approximately six months after fertilization with NEO, no significant stimulating effect on nitrification was detected. This outcome was consistent across different soil types and crops. These findings indicate that the initial stimulatory effects of NEO on nitrification were temporary and attenuated with time after fertilization. While we have observed the transient effects of NEO on nitrification rates, the underlying mechanisms that trigger this boost in nitrification remain to be fully elucidated. Additionally, the role of the taxonomic composition of soil nitrifiers in mediating the response to NEO fertilization requires further investigation.

Declarations

Supplementary Materials: The following supporting information can be downloaded at XXXXX: Figure S1-S25: The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate (NO_2^- + NO_3^-) (blue) at different time points after incubation in different fertilization treatments in the soil samples collected from the cereal and grass fields, incubated as agitated soil slurries in the lab (S1-S8), lab fertilized with different fertilizers and incubated as agitated soil slurries (S9-S20), and lab-fertilized with different fertilizers and incubated as loosely placed, non-agitated soil (S21-S25). The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.; Table S1: Effects of different fertilization treatments on the nitrification rates in different experiments. The Games-Howell pairwise comparison method compares the differences between means at a 95% confidence interval. Averages that do not share a letter are significantly different.

Author Contributions: Conceptualization, H.M., and T.C. and P.D.; methodology, H.M.; T.C. and P.D. software, H.M.; and P.D.; formal analysis, H.M.; and P.D.; investigation, H.M.; and P.D.; resources, H.M.; S.Ø.S.; T.C. and P.D.; data curation, H.M.; and P.D.; writing—original draft preparation, H.M.; S.Ø.S.; P.D.; writing—review and editing, H.M.; S.Ø.S.; T.C. and P.D.; visualization, H.M.; and P.D.; supervision, S.Ø.S.; T.C. and P.D.; project administration, S.Ø.S.; and T.C.; funding acquisition, S.Ø.S.; and T.C. All authors have read and agreed to the published version of the manuscript.

Funding: The Research Council of Norway funded this research (grant number 309640) Plasmabehandlet husdyrgjødsel—gjødselvirkning, miljøpåvirkning og klimagassutslipp, and the APC was funded by Inland Norway University of Applied Sciences (INN).

Data Availability Statement: Data is available at: https://osf.io/7y6n4/

Acknowledgments: Authors would acknowledge Trygve Fredriksen and Mona Mirgeloybayat for their practical assistance in the work. Additionally, the f of the project, the Research Council of Norway, and those who provided us with the required equipment and infrastructure for our experiments: Norwegian Agricultural Extension Service (NLR), Inland Norway University of Applied Sciences (INN), and Norwegian University of Life Sciences (NMBU) are highly acknowledged.

Competing Interest: The authors declare no competing interest. The study's design, data collection, analysis, interpretation; the writing of the article; or the choice to publish the findings were all made independently of the funders.

References

Abraha, A. B., Truter, W. F., Annandale, J. G., & Fessehazion, M. K. (2015). Forage yield and quality response of annual ryegrass (Lolium multiflorum) to different water and nitrogen levels. *African Journal of Range & Forage Science*, *32*(2), 125-131. https://doi.org/10.2989/10220119.2015.1056228

Beeckman, F., Motte, H., & Beeckman, T. (2018). Nitrification in agricultural soils: impact, actors and mitigation. *Curr Opin Biotechnol*, *50*, 166-173. https://doi.org/10.1016/j.copbio.2018.01.014

Bell, C. W., Asao, S., Calderon, F., Wolk, B., & Wallenstein, M. D. (2015). Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biology and Biochemistry*, *85*, 170-182. https://doi.org/10.1016/j.soilbio.2015.03.006

Bi, Q. F., Chen, Q. H., Yang, X. R., Li, H., Zheng, B. X., Zhou, W. W., Liu, X. X., Dai, P. B., Li, K. J., & Lin, X. Y. (2017). Effects of combined application of nitrogen fertilizer and biochar on the nitrification and ammonia oxidizers in an intensive vegetable soil. *AMB Express*, *7*(1), 198. https://doi.org/10.1186/s13568-017-0498-7

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., & Brussaard, L. (2018). Soil quality – A critical review. *Soil Biology and Biochemistry*, *120*, 105-125. https://doi.org/https://doi.org/10.1016/j.soilbio.2018.01.030

Bünemann, E. K., Schwenke, G. D., & Van Zwieten, L. (2006). Impact of agricultural inputs on soil organisms—a review. *Soil Research*, *44*(4). https://doi.org/10.1071/sr05125

C.F. Drury, S. C. H., X.M. Yang. (2007). Nitrification Techniques for Soils. In E. G. G. M.R. Carter (Ed.), *Soil Sampling and Methods of Analysis* (2nd Edition ed.). CRC Press. https://doi.org/https://doi.org/10.1201/9781420005271

Chen, K.-E., Chen, H.-Y., Tseng, C.-S., & Tsay, Y.-F. (2020). Improving nitrogen use efficiency by manipulating nitrate remobilization in plants. *Nature Plants*, *6*(9), 1126-1135. https://doi.org/10.1038/s41477-020-00758-0

Cinar, S., Özkurt, M., & Cetin, R. (2020). Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (Lolium multiflorum l.) in central black sea climatic zone in turkey. *Applied Ecology and Environmental Research*, *18*, 417-432. https://doi.org/10.15666/aeer/1801_417432

Creamer, R. E., Barel, J. M., Bongiorno, G., & Zwetsloot, M. J. (2022). The life of soils: Integrating the who and how of multifunctionality. *Soil Biology and Biochemistry, 166*, 108561. https://doi.org/https://doi.org/10.1016/j.soilbio.2022.108561

DeForest, J. L., & Otuya, R. K. (2020). Soil nitrification increases with elevated phosphorus or soil pH in an acidic mixed mesophytic deciduous forest. *Soil Biology and Biochemistry, 142*, 107716. https://doi.org/10.1016/j.soilbio.2020.107716

Doane, T. A., & Horwáth, W. R. (2011). Spectrophotometric Determination of Nitrate with a Single Reagent. *Analytical Letters, 36*(12), 2713-2722. https://doi.org/10.1081/AL-120024647

Dong, N.-Q., & Lin, H.-X. (2020). Higher yield with less nitrogen fertilizer. *Nature Plants, 6*(9), 1078-1079. https://doi.org/10.1038/s41477-020-00763-3

Döös, B. R. (2002). Population growth and loss of arable land. *Global Environmental Change, 12*(4), 303-311. https://doi.org/10.1016/S0959-3780(02)00043-2

European Commission. (2022). *Sustainable use of nutrients: The common agricultural policy supports farmers in the safe and efficient use of nutrients.* European Union. Retrieved July 18th, 2022 from https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability/low-input-farming/nutrients_en

FAO. (2017). *The future of food and agriculture; trends and challenges.* Food and Agriculture Organization of the United Nations, Rome, Italy. https://www.fao.org/3/i6583e/i6583e.pdf

FAO. (2022, 27.02.2022). *Fertilizers indicators.* FAOSTAT. Retrieved July 18th, 2022 from https://www.fao.org/faostat/en/#data/EF

Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., Sheppard, L. J., Jenkins, A., Grizzetti, B., Galloway, J. N., Vitousek, P., Leach, A., Bouwman, A. F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., & Voss, M. (2013). The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences, 368*(1621), 20130164. https://doi.org/doi:10.1098/rstb.2013.0164

Graves, D. B., Bakken, L. B., Jensen, M. B., & Ingels, R. (2019). Plasma Activated Organic Fertilizer. *Plasma Chemistry and Plasma Processing, 39*(1), 1-19. https://doi.org/10.1007/s11090-018-9944-9

Griess, P. (1858). Vorläufige Notiz über die Einwirkung von salpetriger Säure auf Amidinitro- und Aminitrophenylsäure [Preliminary note on the action of nitrous acid on amidinitro- and aminitrophenylic acid]. *Annalen der Chemie und Pharmacie [Annals of Chemistry and Pharmacy], 106*, 123-125. https://babel.hathitrust.org/cgi/pt?id=njp.32101044011037&view=1up&seq=9

Griffiths, B. S., Römbke, J., Schmelz, R. M., Scheffczyk, A., Faber, J. H., Bloem, J., Pérès, G., Cluzeau, D., Chabbi, A., Suhadolc, M., Sousa, J. P., Martins da Silva, P., Carvalho, F., Mendes, S., Morais, P., Francisco, R., Pereira, C., Bonkowski, M., Geisen, S., . . . Stone, D. (2016). Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecological Indicators, 69*, 213-223. https://doi.org/https://doi.org/10.1016/j.ecolind.2016.04.023

Gu, Y., Wang, Y., Lu, S. *e.*, Xiang, Q., Yu, X., Zhao, K., Zou, L., Chen, Q., Tu, S., & Zhang, X. (2017). Long-term Fertilization Structures Bacterial and Archaeal Communities along Soil Depth Gradient in a Paddy Soil [Original Research]. *Frontiers in Microbiology, 8*. https://doi.org/10.3389/fmicb.2017.01516

Harris, S. L., Thom, E. R., & Clark, D. A. (1996). Effect of high rates of nitrogen fertiliser on perennial ryegrass growth and morphology in grazed dairy pasture in northern New Zealand. *New Zealand Journal of Agricultural Research, 39*(1), 159-169. https://doi.org/10.1080/00288233.1996.9513174

Hu, B. L., Shen, L. D., Xu, X. Y., & Zheng, P. (2011). Anaerobic ammonium oxidation (anammox) in different natural ecosystems. *Biochemical Society Transactions, 39*(6), 1811-1816. https://doi.org/10.1042/bst20110711

Ingels, R., & Graves, D. B. (2015). Improving the Efficiency of Organic Fertilizer and Nitrogen Use via Air Plasma and Distributed Renewable Energy. *Plasma Medicine, 5*(2-4), 257-270. https://doi.org/10.1615/PlasmaMed.2016015763

Kartal, B., van Niftrik, L., Keltjens, J. T., Op den Camp, H. J. M., & Jetten, M. S. M. (2012). Chapter 3 - Anammox—Growth Physiology, Cell Biology, and Metabolism. In R. K. Poole (Ed.), *Advances in Microbial Physiology* (Vol. 60, pp. 211-262). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-398264-3.00003-6

Keeney, D. R., & Nelson, D. W. (1983). Nitrogen—Inorganic Forms. In A. L. Page (Ed.), *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* (pp. 643-698). American Society of Agronomy, Inc., Soil Science Society of America, Inc. https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Kellogg Biological. (2019). *Gravimetric Soil Moisture.* LTER. Retrieved 23.02.2022 from https://lter.kbs.msu.edu/protocols/24

Killham, K. (1990). Nitrification in coniferous forest soils. *Plant and Soil, 128*(1), 31-44. https://doi.org/10.1007/BF00009394

Killham, K. (1998). Soil Microbiology and Biochemistry. By E. A. PAUL and F. E. CLARK. In *New Phytologist* (Vol. 138, pp. 563-566). https://doi.org/10.1111/j.1469-8137.1998.131-4.x

Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., & Garnier, J. (2014). 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters, 9*(10), 105011. https://doi.org/10.1088/1748-9326/9/10/105011

Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data*, *9*(1), 181-192. https://doi.org/10.5194/essd-9-181-2017

Mancus, P. (2007). Nitrogen Fertilizer Dependency and Its Contradictions: A Theoretical Exploration of Social-Ecological Metabolism*. *Rural Sociology*, *72*(2), 269-288. https://doi.org/https://doi.org/10.1526/003601107781170008

Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science*, *277*(5325), 504-509. https://doi.org/10.1126/science.277.5325.504

McAllister, C. H., Beatty, P. H., & Good, A. G. (2012). Engineering nitrogen use efficient crop plants: the current status. *Plant biotechnology journal*, *10*(9), 1011-1025. https://doi.org/10.1111/j.1467-7652.2012.00700.x

Mohanty, S. R., Nagarjuna, M., Parmar, R., Ahirwar, U., Patra, A., Dubey, G., & Kollah, B. (2019). Nitrification Rates Are Affected by Biogenic Nitrate and Volatile Organic Compounds in Agricultural Soils [Original Research]. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00772

Mohanty, S. R., Parihar, M., Dubey, G., Parmar, R., Jain, D., Singh, M., Patra, A., Chaudhari, S. K., & Kollah, B. (2022). Nitrification under the influence of long-term fertilizer application in a tropical vertisol. *Archives of Agronomy and Soil Science*, *68*(4), 516-530. https://doi.org/10.1080/03650340.2020.1843022

Mousavi, H., Cottis, T., Hoff, G., & Solberg, S. Ø. (2022). Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. *Sustainability*, *14*(4), 2005. https://www.mdpi.com/2071-1050/14/4/2005

Mousavi, H., Cottis, T., Pommeresche, R., Dörsch, P., & Solberg, S. Ø. (2022). Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. *Agronomy*, *12*(10), 2314. https://www.mdpi.com/2073-4395/12/10/2314

Mulvaney, R., Khan, S., & Ellsworth, T. (2009). Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. *Journal of environmental quality*, *38*(6), 2295-2314. https://doi.org/10.2134/jeq2008.0527

N2 Applied. (2022). *Nitrogen Enriched Organic fertiliser*. Retrieved July 19th, 2022 from https://n2applied.com/

Nelson, M. B., Martiny, A. C., & Martiny, J. B. H. (2016). Global biogeography of microbial nitrogen-cycling traits in soil. *Proceedings of the National Academy of Sciences*, *113*(29), 8033-8040. https://doi.org/doi:10.1073/pnas.1601070113

Nieder, R., Benbi, D. K., & Scherer, H. W. (2011). Fixation and defixation of ammonium in soils: a review. *Biology and Fertility of Soils*, *47*(1), 1-14. https://doi.org/10.1007/s00374-010-0506-4

Norton, J., & Ouyang, Y. (2019). Controls and Adaptive Management of Nitrification in Agricultural Soils [Review]. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01931

Öhlinger, R., Eibelhuber, A., & Vinzenz, M. (1993). Die Verteilung und Streuung von Bodenenzymaktivitäten in einer einheitlichen Fläche und deren Einfluß auf Probenahme und Analyse [The distribution and scatter of soil enzyme activities in a uniform area and their influence on sampling and analysis]. *Die Bodenkultur - Journal for Land Management, Food and Environment*, *44*(2). https://short.boku.ac.at/wexz4

Ouyang, Y., Evans, S. E., Friesen, M. L., & Tiemann, L. K. (2018). Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: A meta-analysis of field studies. *Soil Biology and Biochemistry*, *127*, 71-78. https://doi.org/https://doi.org/10.1016/j.soilbio.2018.08.024

Prăvălie, R., Patriche, C., Borrelli, P., Panagos, P., Roşca, B., Dumitraşcu, M., Nita, I.-A., Săvulescu, I., Birsan, M.-V., & Bandoc, G. (2021). Arable lands under the pressure of multiple land degradation processes. A global perspective. *Environmental Research*, *194*, 110697. https://doi.org/10.1016/j.envres.2020.110697

Prosser, J. I., & Nicol, G. W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. *Trends in Microbiology*, *20*(11), 523-531. https://doi.org/https://doi.org/10.1016/j.tim.2012.08.001

Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M. C., Koops, H. P., & Wagner, M. (2000). Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. *Appl Environ Microbiol*, *66*(12), 5368-5382. https://doi.org/10.1128/aem.66.12.5368-5382.2000

Raglin, S. S., Soman, C., Ma, Y., & Kent, A. D. (2022). Long Term Influence of Fertility and Rotation on Soil Nitrification Potential and Nitrifier Communities [Original Research]. *Frontiers in Soil Science*, *2*. https://doi.org/10.3389/fsoil.2022.838497

Robertson, G. P., Groffmanand P. M. (2015). 14 - NITROGEN TRANSFORMATIONS. In E. A. Paul (Ed.), *Soil Microbiology, Ecology and Biochemistry*, (Fourth edition ed., pp. 421-426). Academic Press, Burlington, Massachusetts, USA. https://doi.org/10.1016/B978-0-12-415955-6.00014-1

Rütting, T., Schleusner, P., Hink, L., & Prosser, J. I. (2021). The contribution of ammonia-oxidizing archaea and bacteria to gross nitrification under different substrate availability. *Soil Biology and Biochemistry*, *160*, 108353. https://doi.org/https://doi.org/10.1016/j.soilbio.2021.108353

Savci, S. (2012). Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia*, *1*, 287-292. https://doi.org/https://doi.org/10.1016/j.apcbee.2012.03.047

Schinner, F., Öhlinger, R., Kandeler, E., & Margesin, R. (1996). *Methods in Soil Biology* (1 ed.). Springer-Verlag. https://doi.org/10.1007/978-3-642-60966-4

Siebert, J., Sunnemann, M., Auge, H., Berger, S., Cesarz, S., Ciobanu, M., Guerrero-Ramirez, N. R., & Eisenhauer, N. (2019). The effects of drought and nutrient addition on soil organisms vary across taxonomic groups, but are constant across seasons. *Sci Rep*, *9*(1), 639. https://doi.org/10.1038/s41598-018-36777-3

Ste-Marie, C., & Paré, D. (1999). Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biology and Biochemistry*, *31*(11), 1579-1589. https://doi.org/https://doi.org/10.1016/S0038-0717(99)00086-3

Stein, L. Y. (2019). Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology*, *49*, 9-15. https://doi.org/https://doi.org/10.1016/j.cbpa.2018.09.003

Taghizadeh-Hesary, F., Rasoulinezhad, E., & Yoshino, N. (2019). Energy and Food Security: Linkages through Price Volatility. *Energy Policy*, *128*, 796-806. https://doi.org/https://doi.org/10.1016/j.enpol.2018.12.043

Thion, C., & Prosser, J. I. (2014). Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying–rewetting stress. *FEMS Microbiology Ecology*, *90*(2), 380-389. https://doi.org/10.1111/1574-6941.12395

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), 671-677. https://doi.org/10.1038/nature01014

United Nations. (2017). *The World Population Prospects: The 2017 Revision*. United Nations. Retrieved 11 October 2022 from https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100#:~:text=COVID%2D19-,World%20population%20projected%20to%20reach%209.8%20billion%20in%202050%2C%20and,Nations%20report%20being%20

United Nations. (2019). *Meetings Coverage and Press Releases*. UN. Retrieved 11 October 2022 from https://press.un.org/en/2019/sgsm19680.doc.htm#:~:text=Every%20year%2C%20the%20world%20loses,weakened%20resilience%20to%20climate%20chang

United Nations. (2020). *Fertilizers: challenges and solutions*. UN. Retrieved 11 October 2022 from https://www.unep.org/news-and-stories/story/fertilizers-challenges-and-solutions

Upendra, M. S., Rajan, G., & Gautam, P. P. (2019). Nitrogen Fertilization I: Impact on Crop, Soil, and Environment. In R. Everlon Cid & S. Ademar Pereira (Eds.), *Nitrogen Fixation* (pp. Ch. 5). IntechOpen. https://doi.org/10.5772/intechopen.86028

USDA. (2022). *USDA Announces Plans for $250 Million Investment to Support Innovative American-made Fertilizer to give US Farmers more choices in the Marketplace*. The U.S. Department of Agriculture. Retrieved February 12th from https://www.usda.gov/media/press-releases/2022/03/11/usda-announces-plans-250-million-investment-support-innovative#:~:text=Fertilizer%20prices%20have%20more%20than,competition%20in%20the%20fertilizer%20industry.

Wang, F., Chen, S., Wang, Y., Zhang, Y., Hu, C., & Liu, B. (2018). Long-Term Nitrogen Fertilization Elevates the Activity and Abundance of Nitrifying and Denitrifying Microbial Communities in an Upland Soil: Implications for Nitrogen Loss From Intensive Agricultural Systems [Original Research]. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02424

Wang, Y., Ji, H., Wang, R., & Guo, S. (2019). Responses of nitrification and denitrification to nitrogen and phosphorus fertilization: does the intrinsic soil fertility matter? *Plant and Soil*, *440*(1), 443-456. https://doi.org/10.1007/s11104-019-04108-8

Wang, Z. (2010). Griess Diazotization, Z. Wang (Ed.). In *Comprehensive Organic Name Reactions and Reagents* (pp. 1267-1270). https://doi.org/https://doi.org/10.1002/9780470638859.conrr280

Ward, B. B. (2008). Nitrification. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 2511-2518). Academic Press. https://doi.org/https://doi.org/10.1016/B978-008045405-4.00280-9

Watson, C. A., Atkinson, D., Gosling, P., Jackson, L. R., & Rayns, F. W. (2002). Managing soil fertility in organic farming systems. *Soil Use and Management*, *18*(s1), 239-247. https://doi.org/10.1111/j.1475-2743.2002.tb00265.x

Wu, Y., Lu, L., Wang, B., Lin, X., Zhu, J., Cai, Z., Yan, X., & Jia, Z. (2011). Long-Term Field Fertilization Significantly Alters Community Structure of Ammonia-Oxidizing Bacteria rather than Archaea in a Paddy Soil. *Soil Science Society of America Journal*, *75*(4), 1431-1439. https://doi.org/https://doi.org/10.2136/sssaj2010.0434

Yara. (2021). *YaraMila™*. Yara International ASA. Retrieved July 20th 2022 from https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaramila/

yr.no. (2023). *Weather data history* www.yr.no

Zebarth, B. J., Forge, T. A., Goyer, C., & Brin, L. D. (2015). Effect of soil acidification on nitrification in soil. *Canadian Journal of Soil Science*, *95*(4), 359-363. https://doi.org/10.4141/cjss-2015-040

Zwetsloot, M. J., Bongiorno, G., Barel, J. M., di Lonardo, D. P., & Creamer, R. E. (2022). A flexible selection tool for the inclusion of soil biology methods in the assessment of soil multifunctionality. *Soil Biology and Biochemistry*, *166*, 108514. https://doi.org/https://doi.org/10.1016/j.soilbio.2021.108514

Figures

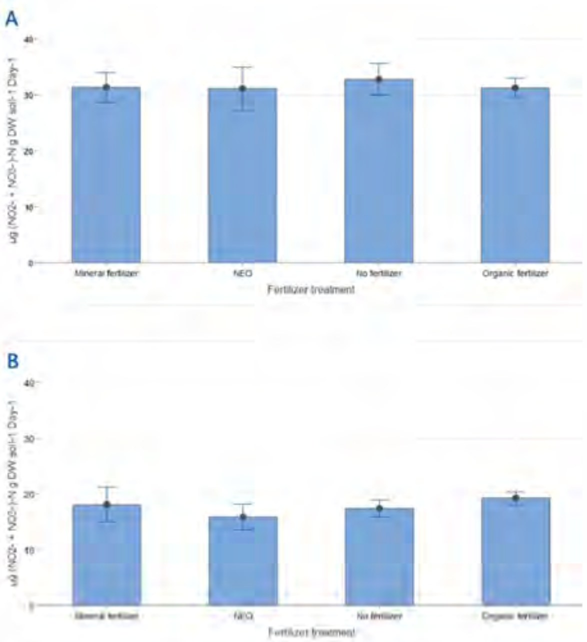


Figure 1

The effect of different fertilization treatments on nitrification potentials in the cereal (A) and the grass field (B) soils collected from the fertilized fields and incubated as agitated soil slurries for 66 hours. NEO = nitrogen-enriched cattle slurry. Organic fertilizer = untreated cattle slurry. Error bars show individual standard deviations (n=4)

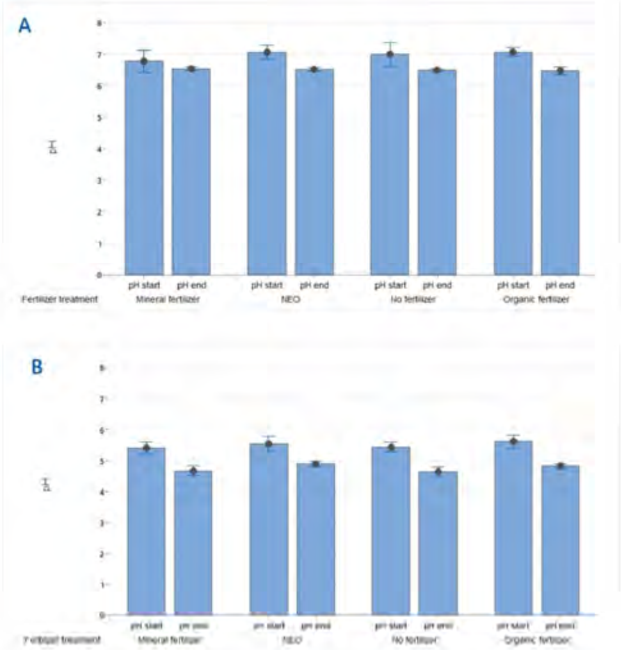


Figure 2

pH changes before and after 66-hour incubation as agitated slurries of cereal (A) and the grass field (B) soils treated with different fertilizers in the field NEO = nitrogen enriched cattle slurry. Organic fertilizer = untreated cattle slurry

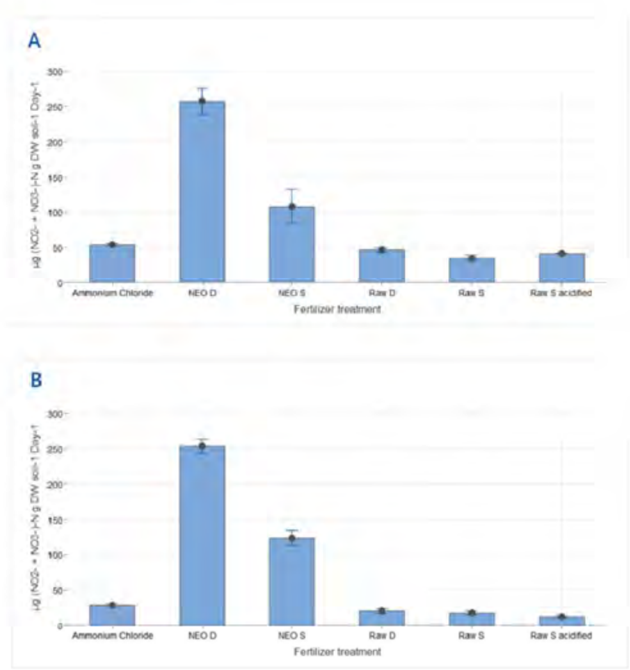


Figure 3

The effect of lab fertilization with different fertilization treatments on nitrification potentials in the cereal (A) and the grass field (B) soils incubated as agitated soil slurries for 43 hours. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries and where Raw S = untreated cattle slurry, Raw D = untreated biogas digestate, NEO S = nitrogen enriched cattle slurry, NEO D = nitrogen enriched biogas digestate, and Raw S acidified = untreated slurry acidified with HCl. Error bars show individual standard deviations (n=3)

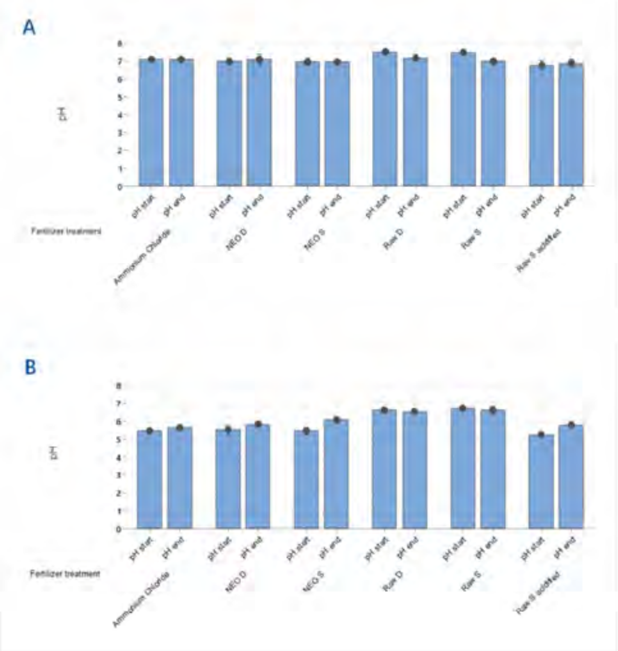


Figure 4

pH changes before and after a 43-hour incubation experiment with agitated soil slurries from the cereal (A) and the grass field (B) amended with different fertilizers in the lab. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries and where Raw S = untreated cattle slurry, Raw D =untreated biogas digestate, NEO S = nitrogen enriched cattle slurry, NEO D = nitrogen enriched biogas digestate, and Raw S acidified = untreated slurry acidified with HCl

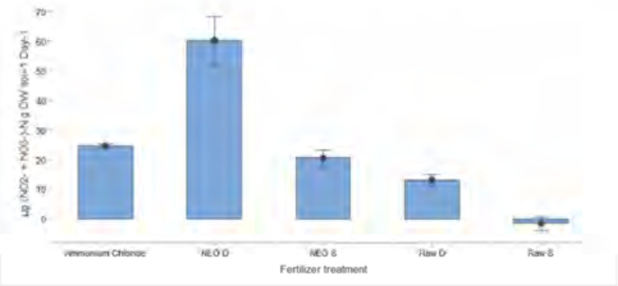


Figure 5

The effect of lab fertilization with different fertilization treatments on nitrification potential in cereal field soil incubated as non-agitated loose soil for 73 hours. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries and where Raw S = untreated cattle slurry, Raw D = untreated biogas digestate, NEO S = nitrogen enriched cattle slurry, NEO D = nitrogen enriched biogas digestate, and Raw S acidified = untreated slurry acidified with HCl. Error bars show individual standard deviations (n=3)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1.docx

Supporting Information:

Nitrogen-Enriched Organic fertilizer (NEO) elevates nitrification rates shortly after application but has no lasting effect on nitrification in agricultural soils

Hesam Mousavi[1,] *, Svein Øivind Solberg[1], Thomas Cottis[1], Peter Dörsch[2]

[1]Faculty of Applied Ecology, Agricultural Sciences, and Biotechnology, Inland Norway University of Applied Sciences, 2418 Elverum, Norway; svein.solberg@inn.no (S.Ø.S.); thomas.cottis@inn.no (T.C.)

[2]Faculty of Environmental Sciences and Natural Resource Management, the Norwegian University of Life Sciences, 1432 Aas, Norway; peter.doersch@nmbu.no (P.D.)

*Correspondence: hesam.mousavi@inn.no (H.M.)

H.M. https://orcid.org/0000-0003-2615-756X; S.Ø.S. https://orcid.org/0000-0002-4491-4483; P.D. https://orcid.org/0000-0002-4916-1839;

Figure S 1 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in unfertilized soil samples collected from the cereal field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

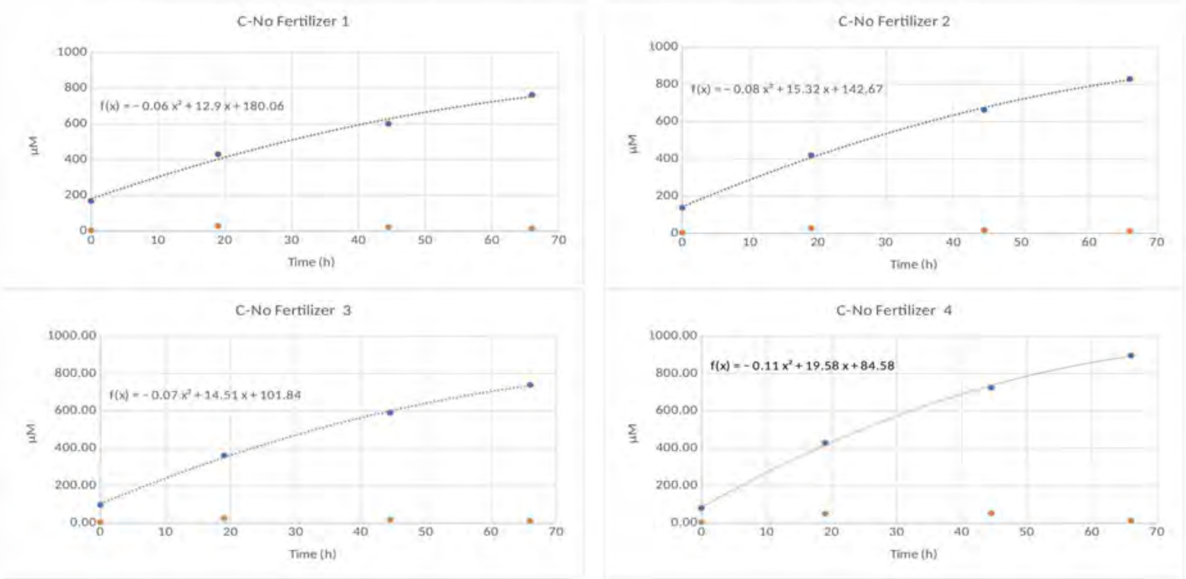


Figure S 2 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in untreated cattle slurry fertilized soil samples collected from the cereal field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

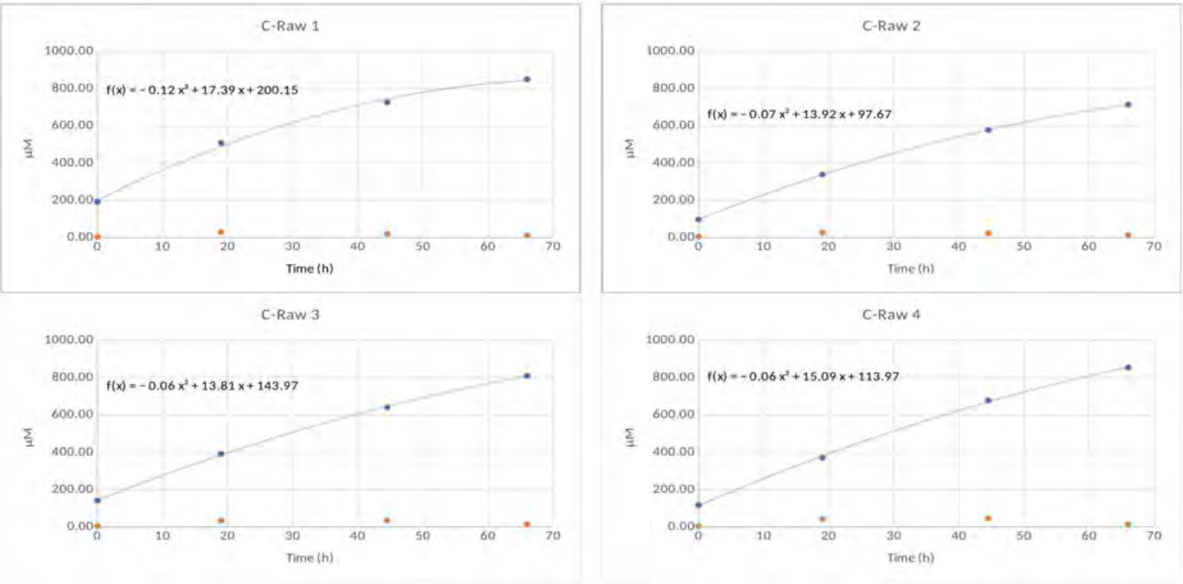


Figure S 3 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in NEO fertilized soil samples collected from the cereal field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

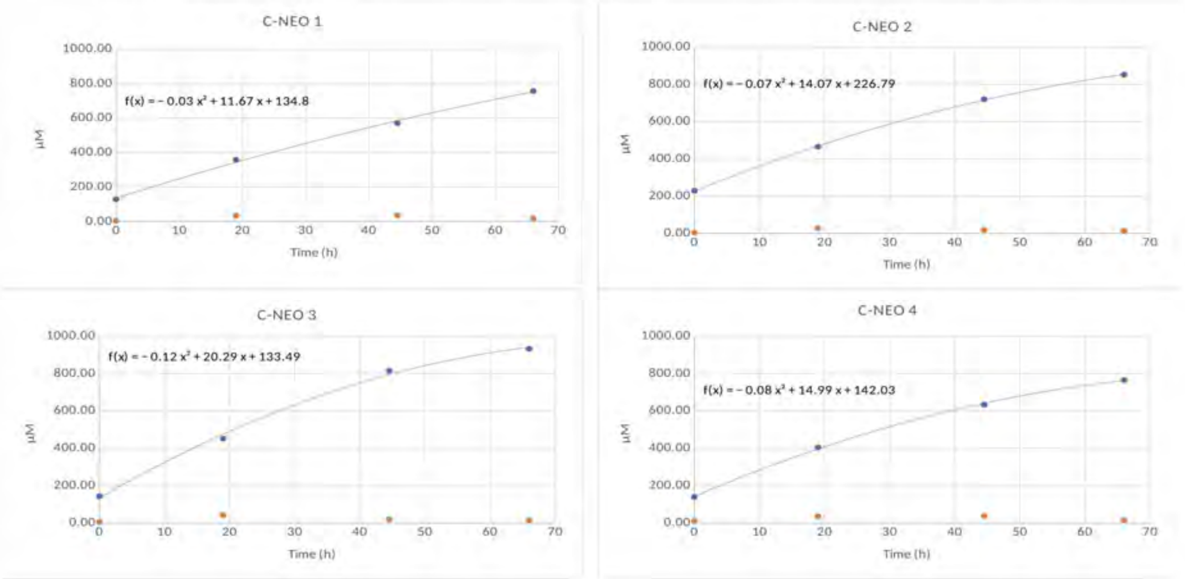


Figure S 4 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in mineral fertilizer fertilized soil samples collected from the cereal field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

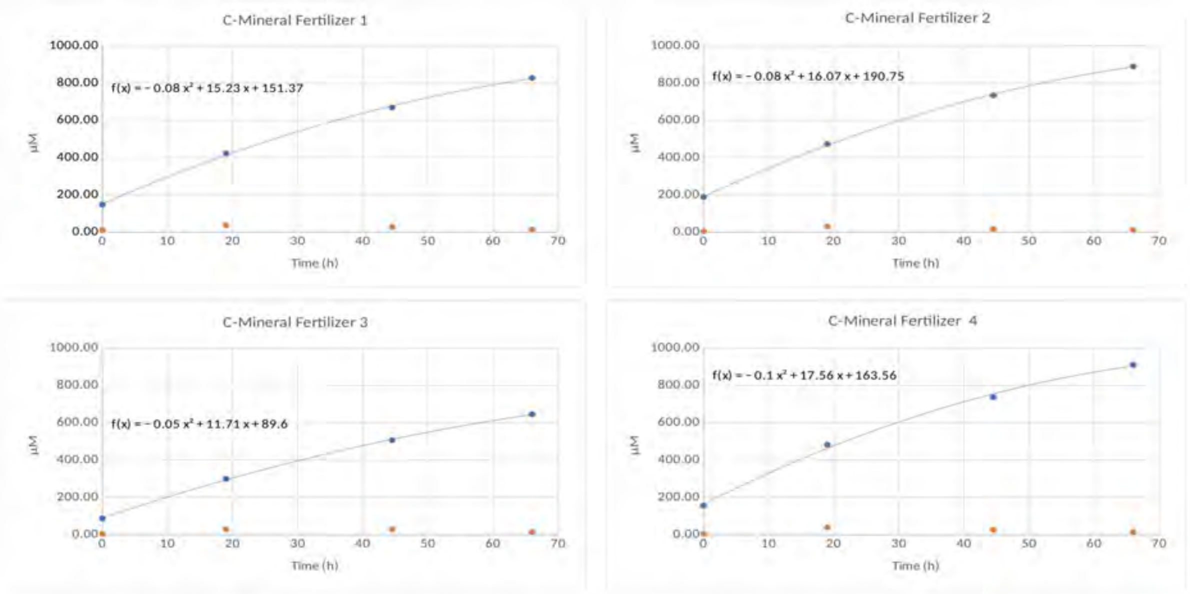


Figure S 5 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in unfertilized soil samples collected from the grass field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

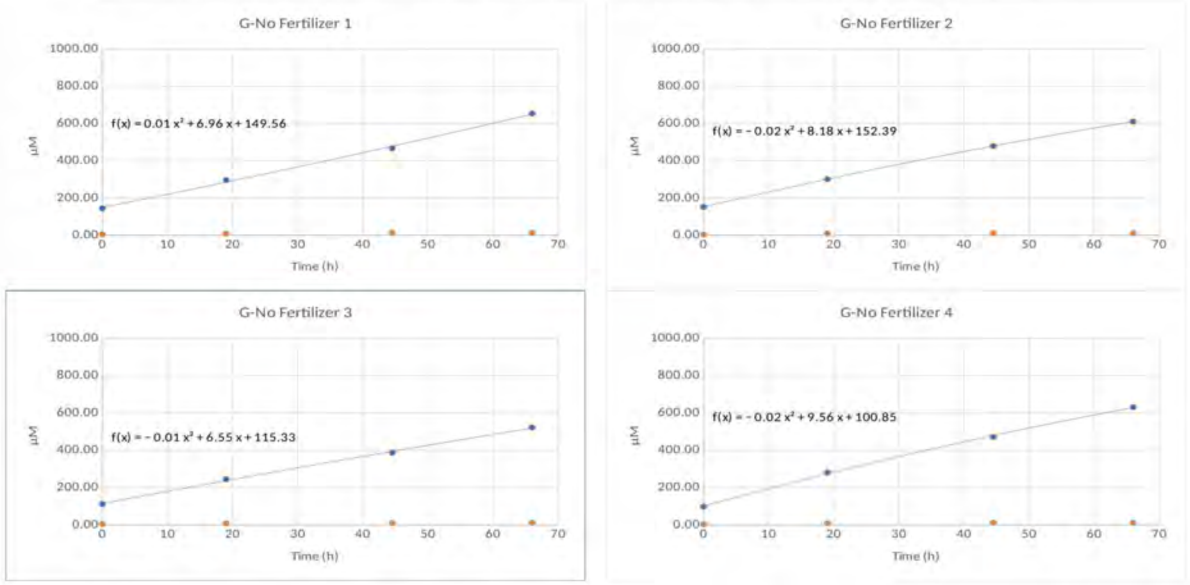


Figure S 6 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in untreated cattle slurry fertilized soil samples collected from the grass field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

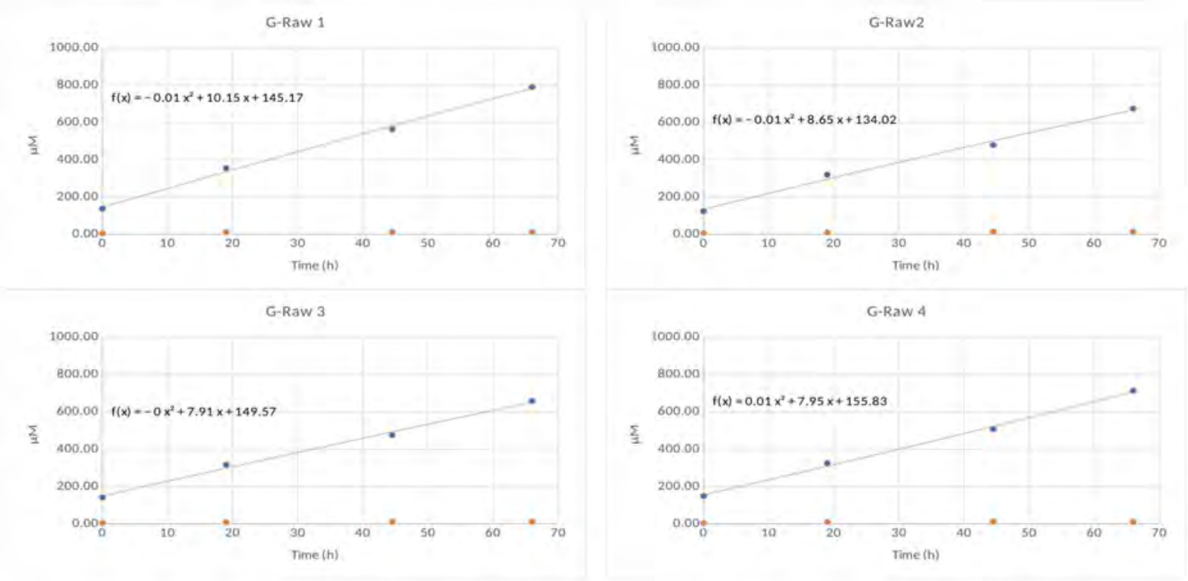


Figure S 7 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in NEO fertilized soil samples collected from the grass field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

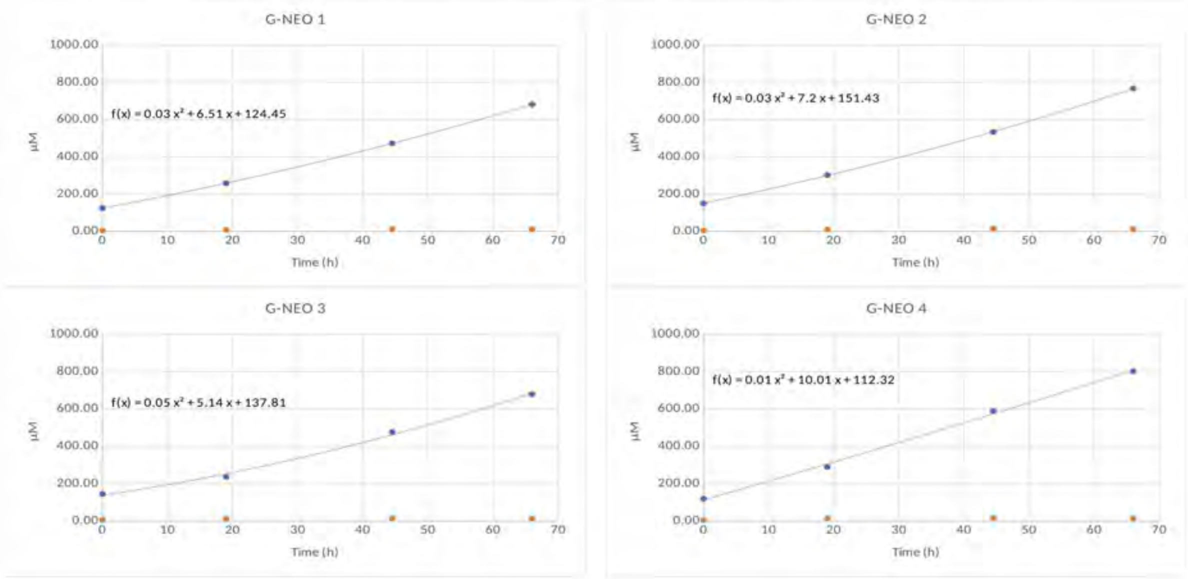


Figure S 8 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in mineral fertilizer fertilized soil samples collected from the grass field and incubated as agitated soil slurries in the lab. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

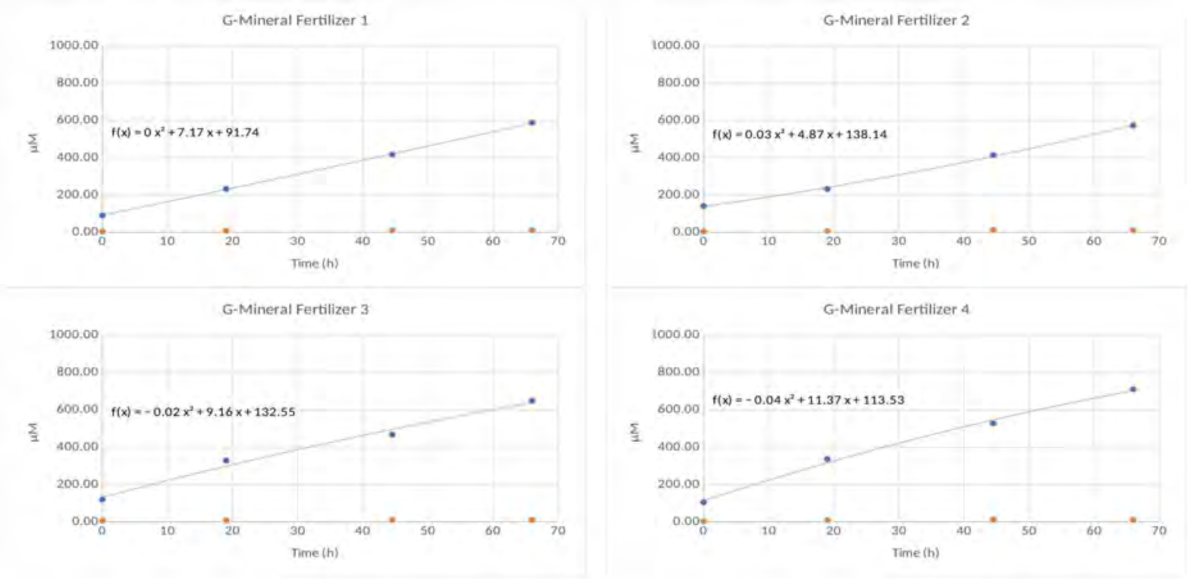


Figure S 9 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in untreated cattle slurry fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

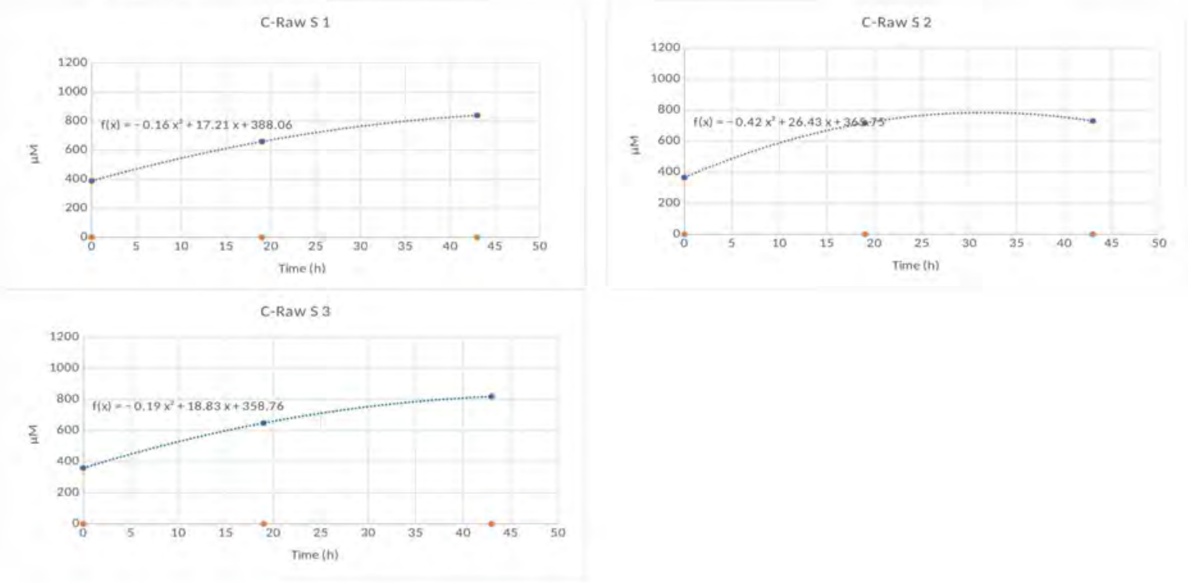


Figure S 10 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate (NO_2^- + NO_3^-) (blue) at different time points after incubation in untreated biogas digestate fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

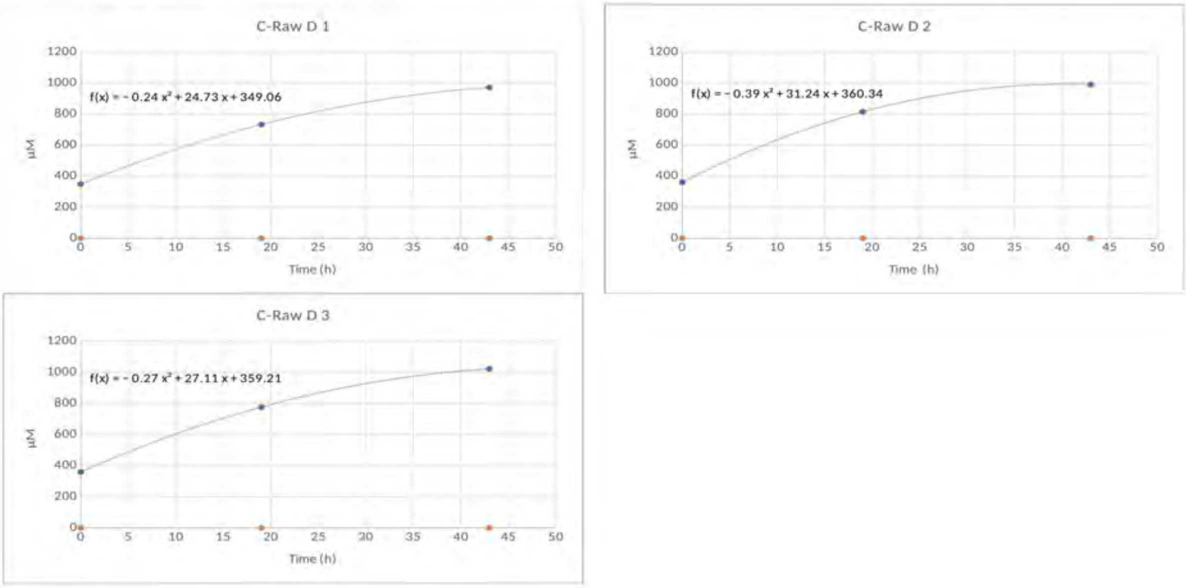


Figure S 11 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate (NO_2^- + NO_3^-) (blue) at different time points after incubation in NEO made from cattle slurry fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

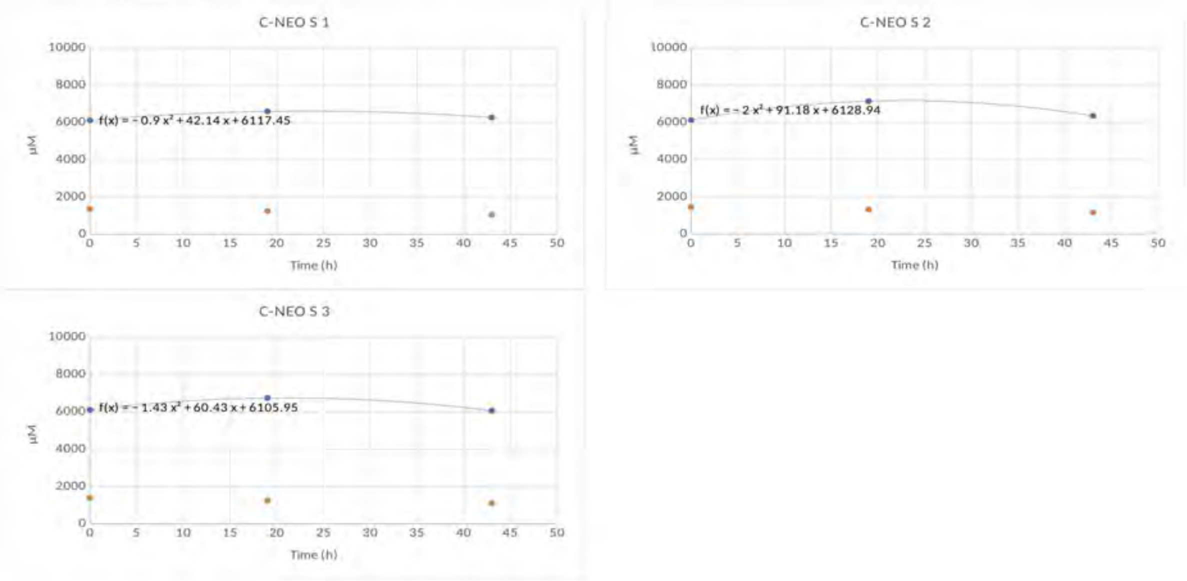


Figure S 12 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in NEO made from biogas digestate fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

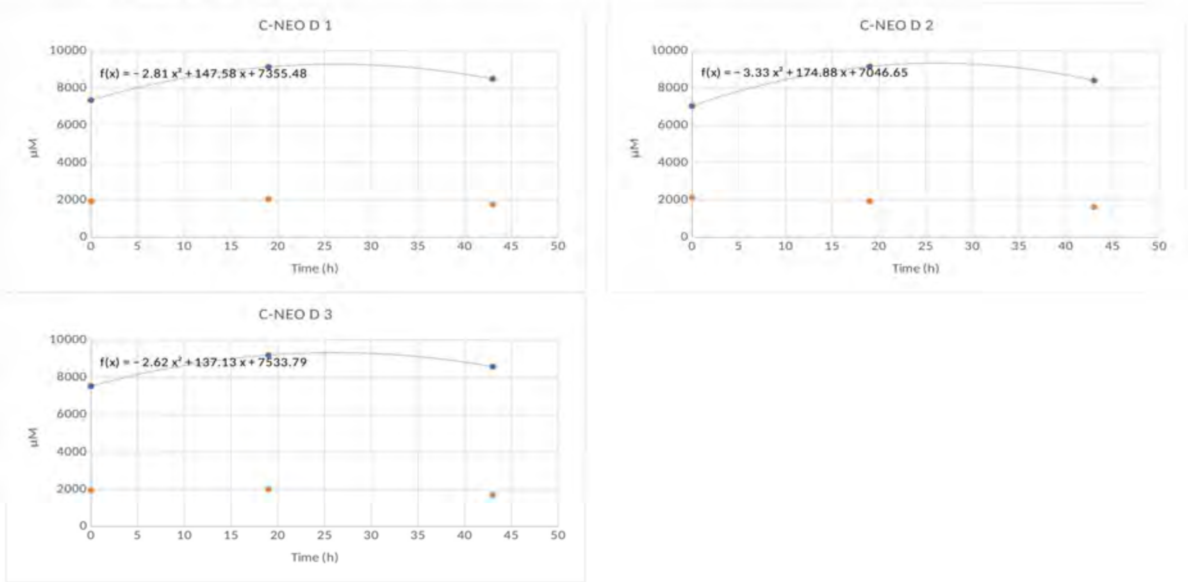


Figure S 13 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in acidified untreated cattle slurry fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

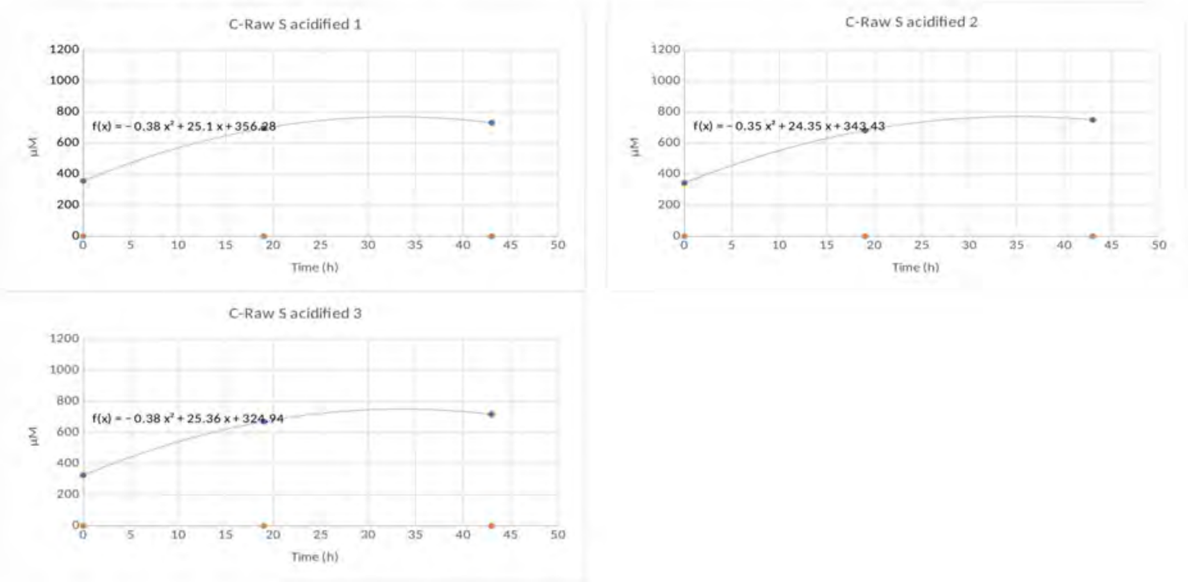


Figure S 14 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate (NO_2^- + NO_3^-) (blue) at different time points after incubation in acidified ammonium chloride fertilized soil samples fertilized in the lab using the cereal field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

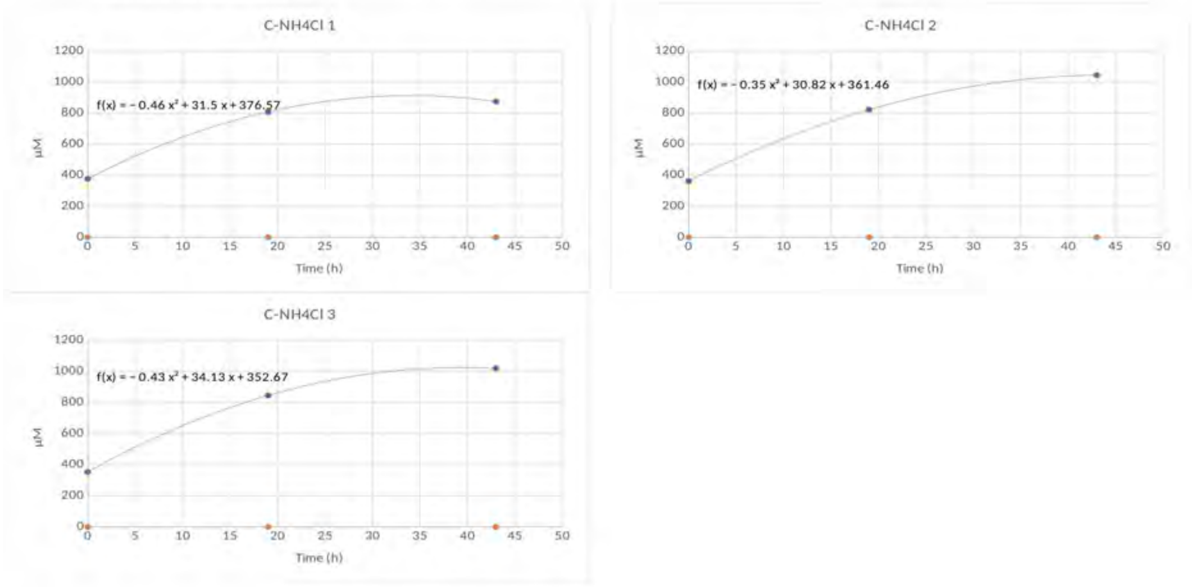


Figure S 15 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate (NO_2^- + NO_3^-) (blue) at different time points after incubation in untreated cattle slurry fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

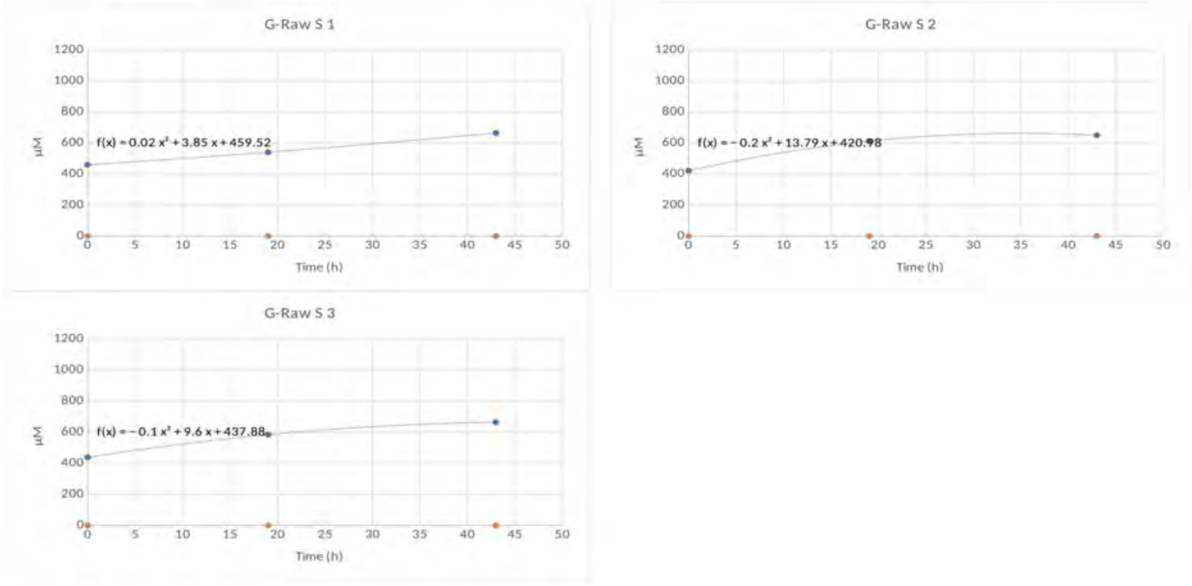


Figure S 16 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in untreated biogas digestate fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

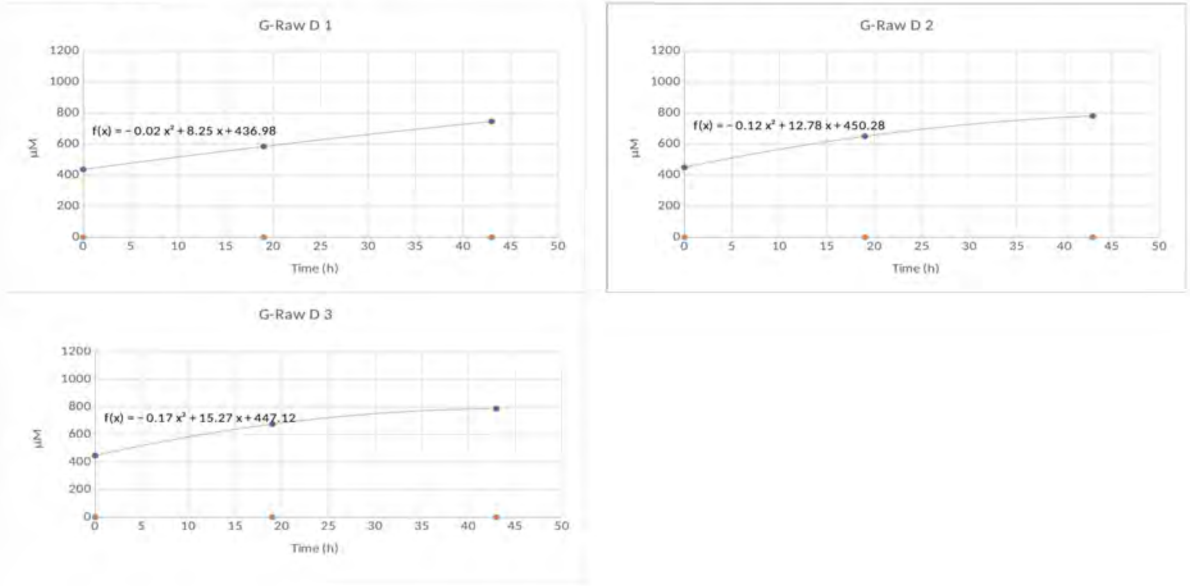


Figure S 17 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in NEO made from cattle slurry fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

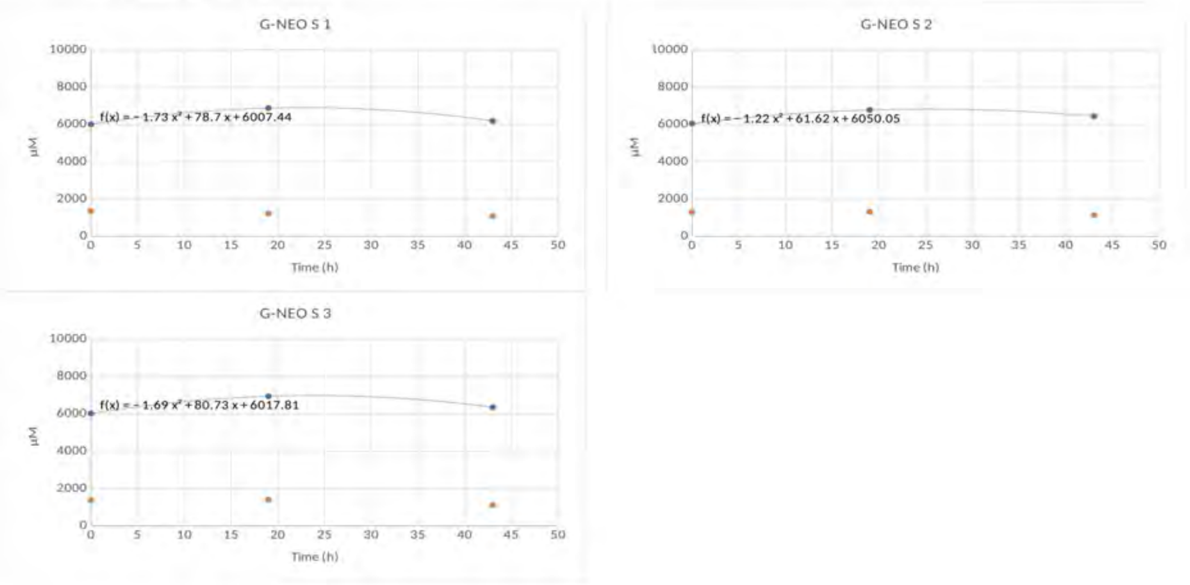


Figure S 18 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in NEO made from biogas digestate fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

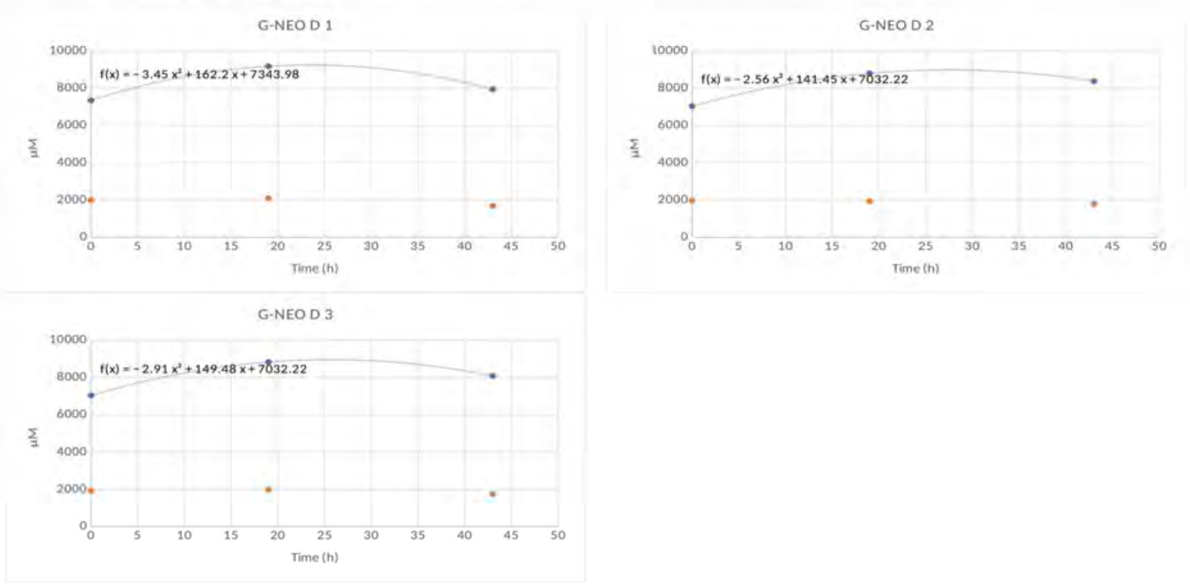


Figure S 19 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in acidified untreated cattle slurry fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

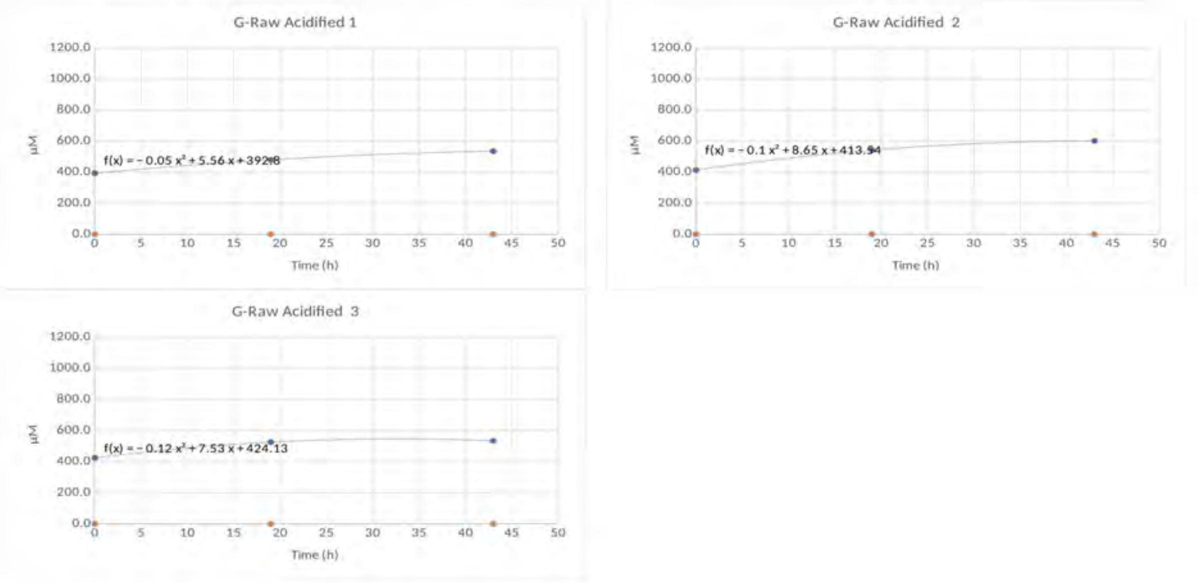


Figure S 20 The amount of nitrite (NO_2^-) (orange) and nitrite + nitrate ($NO_2^- + NO_3^-$) (blue) at different time points after incubation in ammonium chloride fertilized soil samples fertilized in the lab using the grass field soil and incubated as agitated soil slurries. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

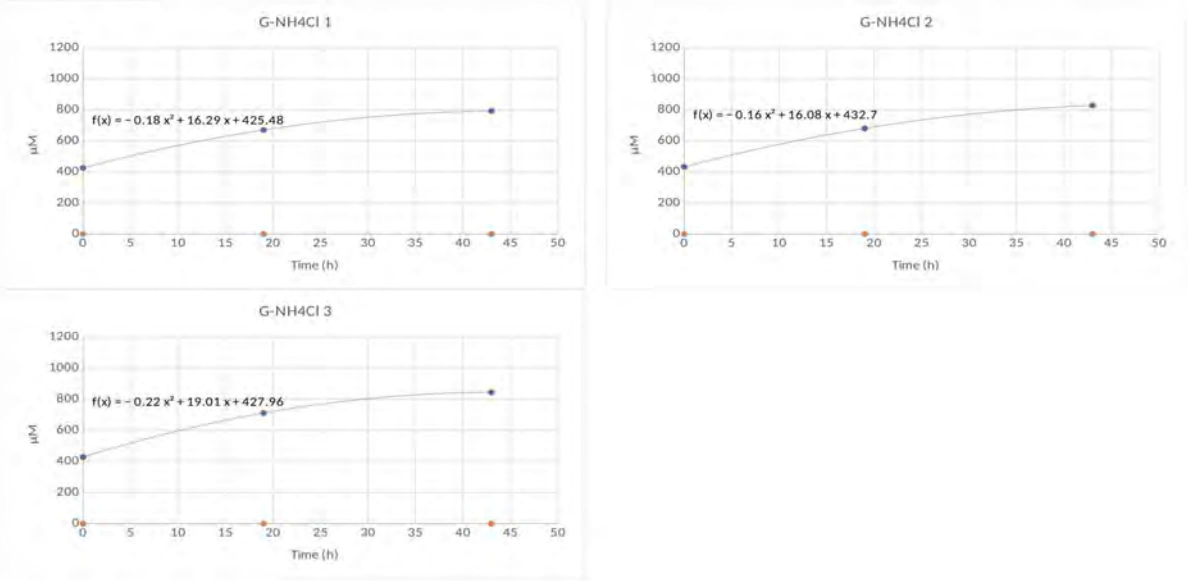


Figure S 21 The amount of nitrite (NO_2^-) (orange), nitrite + nitrate ($NO_2^- + NO_3^-$) (blue), at different time points after incubation in untreated cattle slurry fertilized soil samples fertilized in the lab using the cereal field soil and incubated as non-agitated loosely placed soil. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

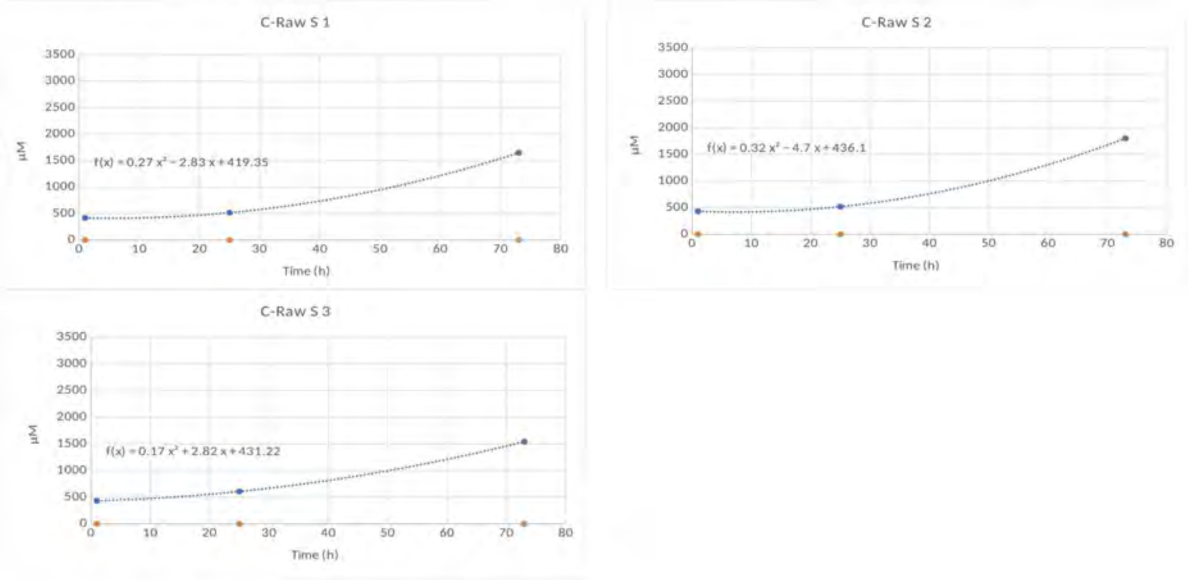


Figure S 22 The amount of nitrite (NO_2^-) (orange), nitrite + nitrate (NO_2^- + NO_3^-) (blue), at different time points after incubation in untreated biogas digestate fertilized soil samples fertilized in the lab using the cereal field soil and incubated as non-agitated loosely placed soil. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

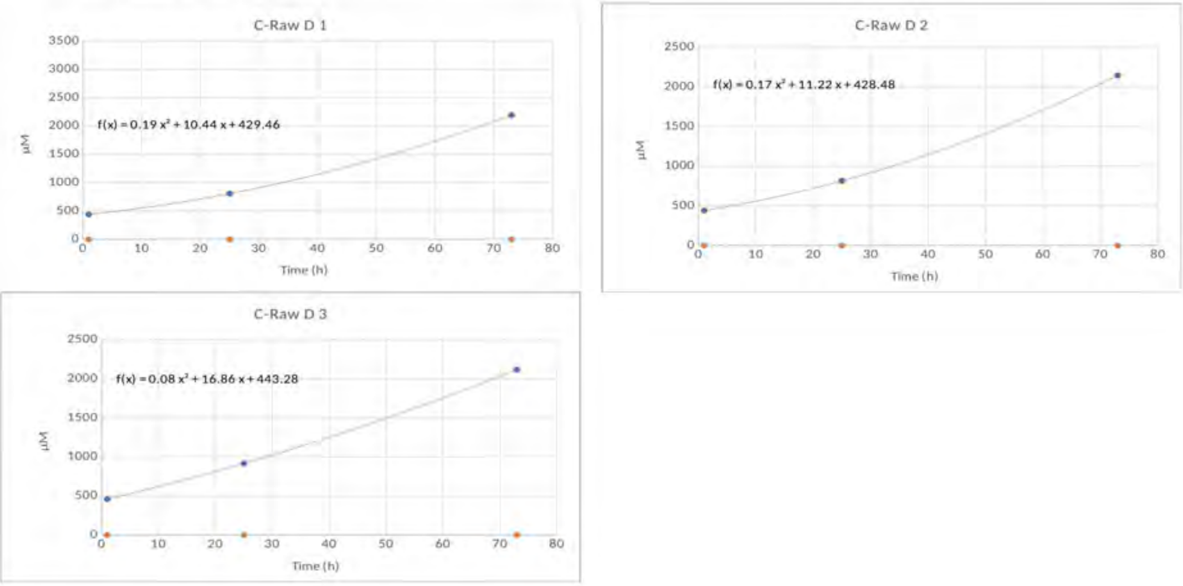


Figure S 23 The amount of nitrite (NO_2^-) (orange), nitrite + nitrate (NO_2^- + NO_3^-) (blue), at different time points after incubation in NEO made from cattle slurry fertilized soil samples fertilized in the lab using the cereal field soil and incubated as non-agitated loosely placed soil. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

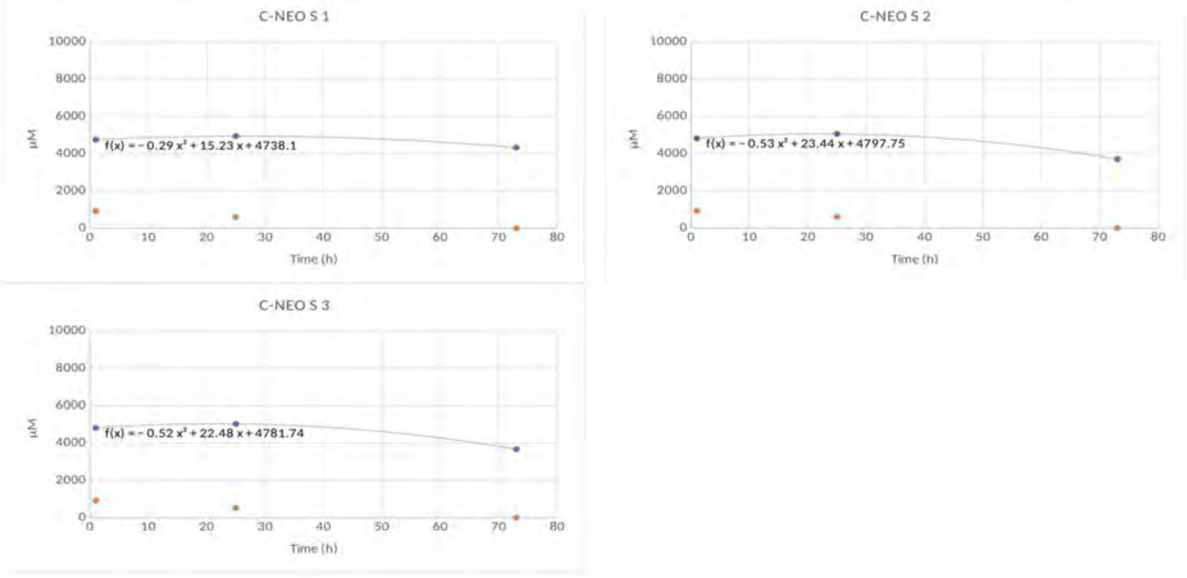


Figure S 24 The amount of nitrite (NO_2^-) (orange), nitrite + nitrate ($NO_2^- + NO_3^-$) (blue), at different time points after incubation in NEO made from biogas digestate fertilized soil samples fertilized in the lab using the cereal field soil and incubated as non-agitated loosely placed soil. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

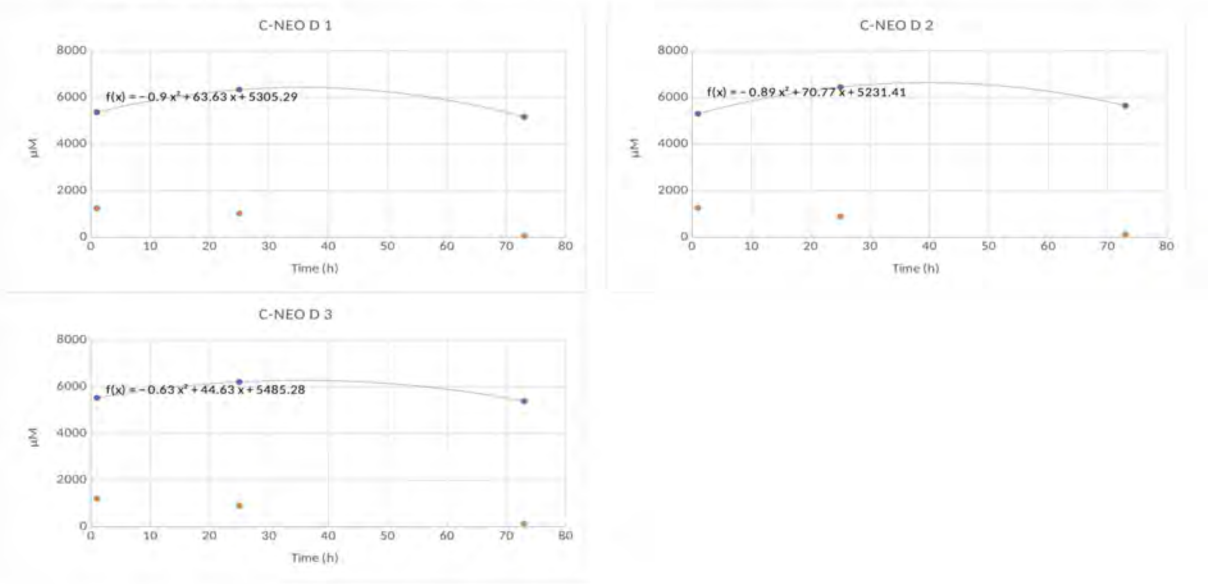


Figure S 25 The amount of nitrite (NO_2^-) (orange), nitrite + nitrate ($NO_2^- + NO_3^-$) (blue), at different time points after incubation in ammonium chloride fertilized soil samples fertilized in the lab using the cereal field soil and incubated as non-agitated loosely placed soil. The polynomial regression equation indicates the growth rate of nitrite + nitrate (nitrification) in the samples over time after incubation.

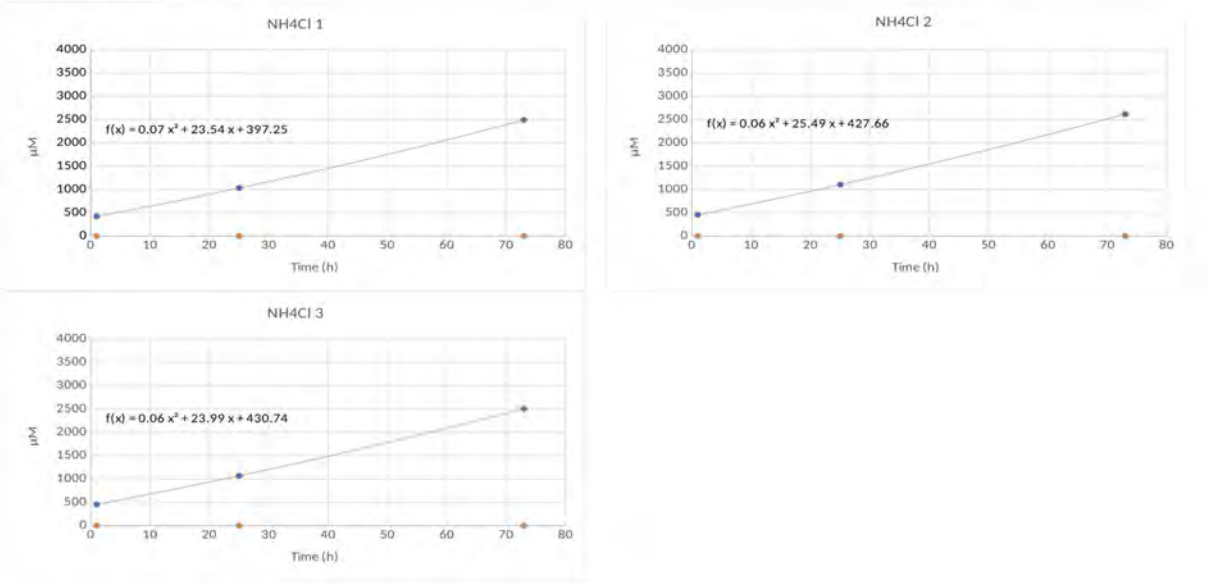


Table 5.1 Effects of different fertilization treatments on the nitrification rates in different experiments. The Games-Howell pairwise comparison method compares the differences between means at a 95% confidence interval. Averages that do not share a letter are significantly different.

Field-fertilized soil (Cereal field)	**µg $(NO_{2-} + NO_{3-})$-N g DW soil^{-1} day^{-1}**	**Grouping**
No fertilizer	32.84	A
Mineral fertilizer	31.41	A
Organic fertilizer	31.37	A
NEO	31.16	A
Field-fertilized soil (Grass field)	**µg $(NO_{2-} + NO_{3-})$-N g DW soil^{-1} day^{-1}**	**Grouping**
Organic fertilizer	19.25	A
Mineral fertilizer	18.08	A
No fertilizer	17.44	A
NEO	15.94	A
Lab-fertilized soil incubated as agitated soil slurries (Cereal field)	**µg $(NO_{2-} + NO_{3-})$-N g DW soil^{-1} day^{-1}**	**Grouping**
NEO D	257.37	A
NEO S	108.49	B
Ammonium Chloride	54.016	C
Raw D	46.52	C
Raw S acidified	41.89	**C**
Raw S	34.98	C
Lab-fertilized soil incubated as agitated soil slurries (Grass field)	**µg $(NO_{2-} + NO_{3-})$-N g DW soil^{-1} day^{-1}**	**Grouping**
NEO D	253.751	A
NEO S	123.788	B
Ammonium Chloride	28.770	C
Raw D	20.327	C
Raw S	17.713	**C**
Raw S acidified	12.174	C
Lab-fertilized soil incubated as non-agitated loosely placed soil	**µg $(NO_{2-} + NO_{3-})$-N g DW soil^{-1} day^{-1}**	**Grouping**
NEO D	60.1540	A
Ammonium Chloride	24.5337	B
NEO S	20.5479	B
Raw D	12.9446	BC
Raw S	-1.5813	**C**

4

agronomy

IMPACT FACTOR 3.7

CITESCORE 5.2

Article

Plasma Treated Cattle Slurry Moderately Increases Cereal Yields

Thomas Cottis, Hesam Mousavi and Svein Øivind Solberg

Special Issue
Cropping Systems and Agronomic Management Practices of Field Crops
Edited by
Dr. Umberto Anastasi and Dr. Aurelio Scavo

MDPI

https://doi.org/10.3390/agronomy13061549

agronomy

Article

Plasma Treated Cattle Slurry Moderately Increases Cereal Yields

Thomas Cottis, Hesam Mousavi * and Svein Øivind Solberg

Faculty of Applied Ecology, Agricultural Sciences, and Biotechnology, Inland Norway University of Applied Sciences, 2418 Elverum, Norway; thomas.cottis@inn.no (T.C.); svein.solberg@inn.no (S.Ø.S.)
* Correspondence: hesam.mousavi@inn.no

Abstract: Plasma treatment offers an approach to enhance the nitrogen (N) content of livestock slurry and biogas digestate, thereby increasing the efficacy of organic fertilizers. This innovative method is used to produce nitrogen-enriched organic fertilizer (NEO) containing a double concentration of plant-available N. Over three years, we conducted a comprehensive study in 14 spring wheat and barley field trials in Norway. The primary objective was to assess and compare the cereal grain yield achieved by applying NEO to other conventional fertilizers. The NEO utilized in our research was derived from the unit developed by the Norwegian company N2 Applied. The results indicated that 120 kg N ha^{-1} in NEO yielded in the same range of cereal grains as 95 kg N ha^{-1} in mineral fertilizer. Moreover, the combination of untreated slurry and 55 kg N ha^{-1} in mineral fertilizer Opti-NS yielded the same as 120 kg N ha^{-1} in NEO. Surprisingly a combination of 12 kg N ha^{-1} in mineral fertilizer at sowing day and 108 kg N ha^{-1} in NEO at the three-leaf stage led to a higher yield in spring wheat than 120 kg N ha^{-1} NEO spread at sowing day in two out of three experimental years. Moreover, applying NEO directly to plants has shown no visible signs of harm. Lastly, filtering the slurry resulted in higher cereal grain yields than the untreated slurry. In conclusion, despite possessing the same N content, utilizing NEO yielded a 15–20% lower cereal grain yield than mineral fertilizer. Nonetheless, 20–30% more yield than the native amount of cattle slurry it derived. However, we have observed an unexplained loss of approximately 17% of the nitrogen in NEO, which does not translate into increased grain yield or nitrogen productivity.

Keywords: agronomy; field crops; fertilization; innovation; wheat; barley; nitrogen

check for updates

Citation: Cottis, T.; Mousavi, H.; Solberg, S.Ø. Plasma Treated Cattle Slurry Moderately Increases Cereal Yields. *Agronomy* **2023**, 13, 1549. https://doi.org/10.3390/agronomy13061549

Academic Editors: Umberto Anastasi and Aurelio Scavo

Received: 4 May 2023
Revised: 30 May 2023
Accepted: 1 June 2023
Published: 2 June 2023

1. Introduction

Global food production systems encounter numerous challenges due to rising food demand, which coincides with population growth [1]. Simultaneously, the detrimental effects of global warming and soil degradation are progressively diminishing production capacity [2]. In this context, agroecosystems face substantial societal pressure to foster sustainable food production [3–6].

The beneficial impact of nitrogen (N) fertilization on plant productivity has been extensively studied and widely acknowledged [7,8]. Moreover, the availability of nitrogen (N) is a fundamental necessity in plant production [9,10], and as such, the utilization of N in agroecosystems has undergone a significant transformation in recent decades [11]. Nevertheless, the excessive application of nitrogen fertilizers can give rise to significant drawbacks and unfavorable outcomes, despite their initial positive effects [12]. Beyond that, the production process of mineral fertilizers results in environmental pollution, disturbance of natural processes, and substantially adverse effects on biodiversity and the climate [13]. This clarifies the necessity of developing sustainable agricultural amendments based on organic principles.

Over the last twelve years, the Norwegian company N2 Applied has developed a unit to enhance the nitrogen content of slurry or digestate, with electricity and air as the only inputs [14–16]. The process uses electrical energy to generate an air plasma, where oxygen and nitrogen combine to form a reactive nitrogen gas. The NOx is subsequently absorbed in the slurry as nitrate and nitrite, enriching the slurry with plant-available nitrogen and reducing the pH. The plasma-treated slurry is termed Nitrogen Enriched Organic fertilizers (NEO). The unit is currently accessible for scientific use and testing by producers, with plans for potential commercial availability in Europe in 2023.

The company reports that their units require 50 kWh of electricity per kg N added to the slurry. Consequently, based on an average addition of 1.65 kg N ton^{-1} slurry, the unit would require 82.5 kWh of electricity per ton of treated slurry. Additionally, the company reports that the N2 Applied unit has a daily 5–8 tons capacity.

Since NEO is a novel product, assessing its impact on plant yield and its effectiveness compared to conventional fertilizers, e.g., mineral fertilizers, cattle slurry, etc., is essential before considering its commercialization. Thus, to accomplish this, we at Inland Norway University of Applied Sciences (INN) conducted comprehensive trials to document and compare the effects of NEO on soil health [17,18] and plant yields in the growing chamber [19] and cereal and grass fields at different locations in Norway over three years (2020 to 2022).

The current study investigates and compares the effects of NEO made from cattle slurry on grain yields of spring barley (*Hordeum vulgare* L.) and spring wheat (*Triticum aestivum* L.), hereafter termed barley and wheat, to other conventional fertilizers used in agriculture. Additionally, we anticipate publishing the results from our grass trials in a forthcoming paper.

Since NEO is a novel product with potentially beneficial gains, its effects on plant yields should be elucidated before introducing it into the global markets. Therefore, this study aims to determine the effects of NEO made from cattle slurry on cereal yields in Norway compared to farmers' alternatives, such as mineral fertilizers and untreated cattle slurry. Therefore, the research questions were: (1) What is the fertilization effect of NEO compared to the other alternatives; and (2) Can NEO be spread at three leaf stage without harming the plants, and if so, would such an application produce a higher yield than NEO spread at the sowing day.

We hypothesized that: (1) NEO could produce the same grain yields as mineral fertilizer, and (2) spreading NEO at the three-leaf stage does not harm the plants but instead boost growth and yield compared to spreading NEO on sowing day.

2. Materials and Methods

2.1. Experimental Design

The experimental design consisted of a randomized complete block design with four replicates, encompassing Series 1 and Series 2. In Series 1, the fertilizer plots measured 10 × 3 m, with a harvested area of 1.5 × 8.5 m within each plot. The larger plot size in Series 1 was necessary to facilitate extensive soil sampling for analyzing soil organisms and overall soil health. In Series 2, the fertilizer plots were smaller, measuring 2.5 × 8 m, with harvest plots at 1.5 × 6.5 m.

The results obtained in 2020 provided indicative evidence supporting the notion that filtered slurry yields positive effects. As a result, the plots receiving the filtered slurry treatment in 2020 were transformed into control treatments with no fertilizers in Series 1 for the 2021 and 2022 trials. Additionally, another finding from 2020 indicated that applying NEO at the three-leaf stage of grain plants resulted in lower yields than NEO applied on sowing day. Consequently, a separate series of trials in Series 2 was designed, omitting the NEO application at the three-leaf stage treatment. Furthermore, the N-level treatments in mineral fertilizers were increased to assess the nitrogen effect of NEO better.

2.2. Trials Location

The field trials were located at four representative areas for cereal production in Norway: 1. Tønsberg; 2. Årnes; 3. Hamar; and 4. Stjørdal (Figure 1). Details on the locations and soil types are provided in Table 1.

Figure 1. The map showing the locations in the southern part of Norway, where the field trials were conducted in 2020, 2021, and 2022: Tønsberg 1, Årnes 2, Hamar 3, and Stjørdal 4.

Table 1. The trial numbers, location coordinates, and soil quality information at the trial sites. The barley and wheat were all spring-sown types.

Series	Trial	Location	Crops, Varieties, and Years	Detailed Location and Coordinates	Soil Type and Key Soil Parameters
1	1	3	Barley' Salome' 2020, Wheat 'Betong' 2021, Barley' Bente' 2022	3 km east of Hamar (60.81830° N. 011.17968° E)	Loam. 4.5% organic. pH 7.4
1	2	3	Wheat 'Mirakel' 2020, Barley' Anita' 2021, Wheat 'Betong' 2022	3 km east of Hamar (60.81830° N. 011.17968° E)	Loam. 4.5% organic. pH 7.4
1	3	2	Wheat 'Helmi' 2021	3 km west of Årnes (60.12604° N. 11.39471° E)	Silt loam. 4.0% organic. pH 6.0
1	4	2	Barley 'Brage' 2021	3 km west of Årnes (60.12604° N. 11.39471° E)	Silt loam. 4.0% organic. pH 6.0
2	5	1	Wheat 'Betong' 2021	5 km west of Tønsberg (59.294937° N. 10.318813° E)	Silt loam. 6.5% organic. pH 6.2
2	5	1	Wheat 'Betong' 2022	15 km north of Tønsberg (59.384537° N. 10.232651° E)	Silt loam. 4.8% organic. pH 6.9
2	6	4	Barley 'Thermus' 2021	4 km north of Stjørdal (70.41109° N. 59.3647° E)	Loam. 2.7% organic. pH 6.1
2	6	4	Barley 'Thermus' 2022	4 km north of Stjørdal (70.37496° N. 59.7733° E)	Loam. 2.7% organic. pH 6.1

2.3. *Fertilizers*

In the trials, we used the following fertilizers:

- Untreated slurry: Cattle Slurry from the Norwegian University of Life Sciences farm.
- NEO (Nitrogen Enriched organic fertilizer): This is the same slurry as «Untreated slurry» processed through the N2 Applied unit. The available nitrogen in NEO is around 50% ammonia, 30% nitrate, and 20% nitrite, and the acidity is down to around pH 5.2. The relative levels of nitrate and nitrite vary quite a lot. See Table 2.
- Mineral fertilizer 18-3-15: A commercially available mineral fertilizer produced by Yara [20] with 18% nitrogen (N), 3% phosphorus (P), and 15% potassium (K). The 18% N consists of slightly more ammonia than nitrate. This fertilizer was chosen due to the similarities in plant available nutrients to NEO.

- Mineral fertilizer Opti-NS (27-0-0) [21]: This is an N fertilizer combined with sulfur (S) (3.6%), where the N consists of equal amounts of ammonia and nitrate.

Table 2. Amounts of mineral N, ammonia N, nitrate N, nitrite N, total N (kg N ton^{-1}), and pH in the NEO and untreated cattle slurry used over the three years (average over several analyses per year).

Fertilizer and Year	N-Min (kg ton^{-1})	NH^{4+} (kg ton^{-1})	NO^{3-} (kg ton^{-1})	NO^{2-} (kg ton^{-1})	Total N (kg ton^{-1})	pH
NEO 2020	3.4	1.68	1.24	0.52	4.7	5.3
Untreated 2020	1.7	1.7	0	0	2.8	7.1
NEO 2021	3.2	1.5	0.92	0.8	4.38	5.59
Untreated 2021	1.5	1.5	0	0	2.68	7.17
NEO 2022	3.55	1.66	1.19	0.69	Not analyzed	5.15
Untreated 2022	1.75	1.7	0	0	Not analyzed	7.35

During the production process in N2-Applied's plasma reactor, the untreated slurry undergoes filtering to remove solid particles larger than 5 mm using a screw press, which reduces the original volume by 10%. As a result, the filtered material has a consistency similar to soft coarse peat. This filtering, combined with the plasma treatment, transforms the liquid fertilizer into NEO, which exhibits enhanced soil permeation compared to the untreated liquid slurry.

To determine the appropriate quantities of NEO and other fertilizers for the different trial plots, the company sent samples to AnalyTech Environmental Laboratory in Denmark. In 2021 and 2022, the analysis was conducted on the untreated manure and the pre-produced NEO two weeks before their application in the experimental sites. Table 2 provides the nitrogen and pH values of NEO and untreated slurry for 2020, 2021, and 2022.

In 2020, N2 Applied conducted a test production of NEO in March and sent samples for nitrogen content testing to the Danish lab. The fertilizer amounts for the 2020 trials were calculated based on the results. Unfortunately, an error occurred during the production of NEO intended for the field trials, resulting in lower nitrogen content than initially calculated. As a result, the results from the 2020 trials remain valid but cannot be directly compared. Instead, they serve as supporting material for the results obtained in 2021 and 2022.

The primary objective of our studies was to assess the impact of NEO on crop yield in comparison to other farmer alternatives. Therefore, we established a baseline of 120 kg N ha^{-1} for both wheat and barley, considering it as a typical level for barley in Norway's grain regions, albeit slightly lower than what is commonly used for spring wheat. To achieve the desired nitrogen level of 120 kg per hectare, approximately 40 tons of cattle slurry were processed through the N2 Applied's plasma process unit after filtering. The process converted 40 tons of cattle slurry into 37 tons of NEO containing 120 kg of nitrogen.

In 2020, we conducted two trials in series 1. Unfortunately, due to the abovementioned production error, the nitrogen content in the cattle slurry-based treatments differed in 2020 compared to 2021 and 2022. Table 3 presents the treatments labeled (bold) in series 1 and 2 over three years.

Filtered slurry and NEO have different N contents from year to year. Considering this, adjustments were made to keep the nitrogen content per hectare constant from year to year as the most decisive factor.

In 2021 the N-content in NEO was 3.2 kg N ton^{-1}, and we aimed for fertilization with 120 kg N ha^{-1} in NEO, accordingly 37.5 tons ha^{-1}. As mentioned, 10% of cattle slurry is filtered through NEO production. Thus, the farmers' alternative is to spread 41 tons ha^{-1} of untreated slurry. In 2021 the N-content in the untreated slurry was 1.5 kg N ton^{-1}. This year, applying 41 tons ha^{-1} of untreated slurry to the trial plots provided 61.5 kg N/ha^{-1}. In 2022 the NEO had 3.55 kg N ton^{-1}, resulting in 34 tons ha^{-1}

of NEO reaching 120 kg N ha^{-1}. The untreated had 1.75 kg N ton^{-1}, and with a 10% higher volume than NEO, we applied 37 tons ha^{-1} of untreated slurry—resulting in 65 kg N ha^{-1}. With this clarification, we used 65 kg N ha^{-1} in the graph labels for the untreated slurry for 2021 and 2022.

Table 3. The treatments and their labels (bold text) used in our trials.

Treatments in Series 1 in 2020:	**Treatments in Series 1 in 2021 and 2022:**	**Treatments in Series 2 in 2021 and 2022:**
MaF51: 51 kg N ha^{-1} in Filtered untreated slurry.	NoF: No fertilizer	NoF: No fertilizer
Ma56: 56 kg N ha^{-1} in untreated slurry	Ma65: 65 kg N ha^{-1} in untreated slurry (manure).	NEO120: 120 kg N ha^{-1} in NEO
NEO102: 102 kg N ha^{-1} in NEO	NEO120: 120 kg N ha^{-1} in NEO	Ma65: 65 kg N ha^{-1} in untreated slurry.
MiNEO 104: 12 kg N ha^{-1} in mineral fertilizer 18-3-15 applied to the trial plots before sowing and 92 kg N/ha^{-1} in NEO at Zadoks GS13 three leaves stage.	MiNEO 120: 12 kg N ha^{-1} in mineral fertilizer 18-3-15 applied to the trial plots before sowing and 108 kg N/ha^{-1} in NEO at Zadoks GS13 three leaves stage.	MaMi120: 65 kg N ha^{-1} in untreated slurry and 55 kg N ha^{-1} in mineral fertilizer Opti-NS.
Mi51: 51 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi65: 65 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi30: 30 kg N ha^{-1} in mineral fertilizer 18-3-15
Mi91: 91 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi91: 91 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi55: 55 kg N ha^{-1} in mineral fertilizer 18-3-15
Mi123: 123 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi120: 120 kg N ha^{-1} in mineral fertilizer 18-3-15	Mi80: 80 kg N ha^{-1} in mineral fertilizer 18-3-15
MaMi123: 56 kg N ha^{-1} in untreated slurry combined with 6.7 kg N ha^{-1} in mineral fertilizer Opti-NS	MaMi120: 65 kg N ha^{-1} in untreated slurry combined with 55 kg N ha^{-1} in mineral fertilizer Opti-NS	Mi105: 105 kg N ha^{-1} in mineral fertilizer 18-3-15
		Mi120: 120 kg N ha^{-1} in mineral fertilizer 18-3-15

It is also necessary to clarify a point regarding the mineral fertilizer plus NEO treatment (MiNEO120). In earlier testing of NEO, the N2 Applied company had experienced that NEO could be applied to cereals after germination. Therefore, we agreed to test this in series 1 by forming the MiNEO120 treatment, where we applied 12 kg N ha^{-1} in mineral fertilizer Yara Mila complete fertilizer 18-3-15 (Yara, Oslo, Norway) to the trial plots before sowing, combined with 108 kg N ha^{-1} in NEO applied at three leaves stage Zadoks GS13 [22]. All the other treatments were applied on sowing day by spreading the fertilizers on the trial plots and mixing them into the soil using a disc harrow. The grain was sown a few hours after fertilization.

2.4. Weather Conditions

Table 4 presents May's average temperature, precipitation, and corresponding average values in all trial locations over 2020–2022. Series 1 had trials in Hamar and Årnes. In 2020, Hamar was 1.4 °C colder than average and had less than half of the normal precipitation. In 2021, Hamar and Årnes had a normal average temperature but about 20% more precipitation than normal. In 2022, Hamar had a normal average temperature but a dry month of May with 23.2 mm less precipitation than the normal 55 mm. The trials in Series 2 were in Tønsberg and Stjørdal. Tønsberg had 24 mm more rain than average in 2021 and about half the normal precipitation in 2022. Stjørdal had a dry month in May, with half the normal precipitation in 2021 and average rainfall in 2022 [23].

Table 4. Average temperatures, normal temperatures, precipitation, and normal precipitation for the grain trial locations over the years 2020–2022.

Year	Location	Average Temperature (°C)	Normal Temperature (°C)	Average Precipitation (mm)	Normal Precipitation (mm)
2020	Hamar	8.5	9.9	23	55
2021	Tønsberg	9.9	10.8	95.1	71
2021	Årnes	9.3	10.2	88.4	59
2021	Hamar	9.5	9.9	77.9	55
2021	Stjørdal	9.6	9.0	33.1	63
2022	Tønsberg	11.4	10.8	36.5	71
2022	Hamar	9.8	9.9	31.8	55
2022	Stjørdal	9.6	9.0	72.6	63

2.5. *Data Handling, Statistics, and Analysis*

Field trial data were first analyzed using ANOVA and Duncan's multiple-range tests of the means. Then, the N effect of NEO was calculated against the nitrogen effect of mineral fertilizer. This was possible as we included a mineral fertilization ladder ranging from 0 kg N-min ha^{-1} to 120 kg N-min ha^{-1}. Next, a linear regression model expressed the relationship between N provided in mineral fertilization (x axis) and grain yield (y axis). The same was done for the N yield data. The regression equations were then used to calculate the N effect of 120 kg N-min ha^{-1} provided in NEO based on yield and N yield data, respectively. This procedure was repeated for each of the trials and finally across all trials, with 95% confidence intervals. Statistical analyses were done in SPSS 28 software (© 2023 IBM (New York, NY, USA). Excel (© 2023 Microsoft (Seattle, WA, USA), and Minitab 21 (2023 Minitab, LLC (State College, PA, USA)), were used for the graphics. Finally, we analyzed samples from all the trial plots for N percentage with the Dumas method to find the Nitrogen yields.

3. Results

3.1. *Barley and Wheat Grain and Nitrogen Yield—Series 1 2020*

In 2020, when examining barley grain yield (Figure 2A), it was found that NEO102 produced a yield equivalent to that of MiNEO104, Mi91, and MaMi123. However, Mi123 exhibited a significantly higher yield than all other treatments. Additionally, MaF51 demonstrated a yield of 448 kg ha^{-1}, which was significantly higher than that of Ma56.

When considering wheat grain yield (Figure 2A), it was observed that NEO102 yielded significantly more (469 kg ha^{-1}) compared to MiNEO104 while producing a yield similar to that of Mi91 and MaMi123. However, once again, Mi123 displayed a significantly higher yield than the rest of the treatments. Notably, MaF51 demonstrated a significantly higher yield of 756 kg ha^{-1}, surpassing that of Ma56.

The trend in nitrogen yield for barley and wheat (Figure 2B) followed a similar pattern to grain yield; however, the differences between the treatments became more pronounced.

3.2. *Barley and Wheat Grain and Nitrogen Yield—Series One, 2021 and 2022*

Here, we present the results from three separate trials conducted in barley and wheat as part of Series one in 2021 and 2022. We have analyzed the data separately for grain yield and nitrogen yield.

Regarding barley grain yield (Figure 3A) among the treatments, Mi120 demonstrated the highest yield, surpassing MiNEO120, NEO120, and MaMi120 by 586 kg ha^{-1}, 610 kg ha^{-1}, and 793 kg ha^{-1}, respectively. Notably, MiNEO120 and NEO120 yielded alike, with both treatments significantly outperforming Ma65 and falling within the range of Mi91.

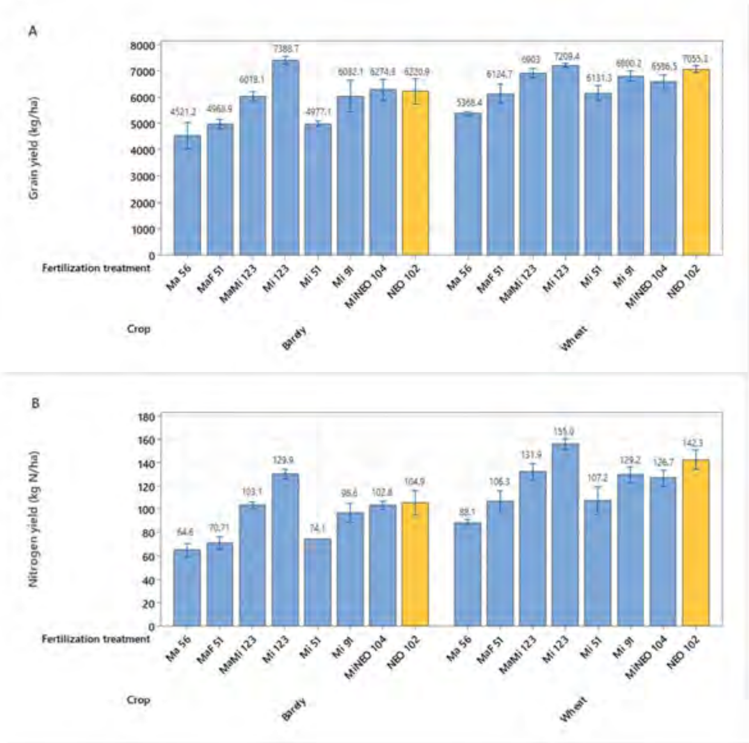


Figure 2. (**A**) Grain yield (15% water content) in kg ha^{-1} and (**B**) nitrogen yield in kg N ha^{-1} ± standard error of the means. Results from the initial trials in 2020 with one trial in barley (**left**) and one in spring wheat (**right**). The NEO102 kg N ha^{-1} treatment stands out from other treatments with its distinct yellow coloration.

Furthermore, regarding wheat grain yield (Figure 3A), unlike the barley results, MiNEO120 yielded higher than other treatments; the difference was insignificant to Mi120, NEO120, and MaMi120. NEO120 yielded in the same range as Mi91 but significantly surpassed Ma65.

Regarding nitrogen barley yield (Figure 3B), the pattern observed mirrored that of grain yield, displaying similar trends across the treatments. However, considering the nitrogen wheat yield (Figure 3B), MiNEO120 exhibited the highest nitrogen yield, reaching 106.1 kg N ha^{-1}. This result was significantly higher than both NEO120 and MaMi120, and it exceeded Mi120 by an additional 8.7 kg N ha^{-1}, although the latter difference was insignificant.

3.3. Barley and Wheat Grain and Nitrogen Yield—Series Two, 2021–2022

In series two of the experiments conducted in 2021 and 2022, the barley grain yield (Figure 4A) of MaMi120 was 5064 kg ha^{-1}, which was similar to the yield of Mi120. However, Mi120 yielded only 170 kg ha^{-1} higher than NEO120, and the difference was not statistically significant. On the other hand, NEO120 produced a grain yield that fell between the yields of Mi105 and Mi120, with a significantly higher yield of 646 kg ha^{-1} compared to Ma65.

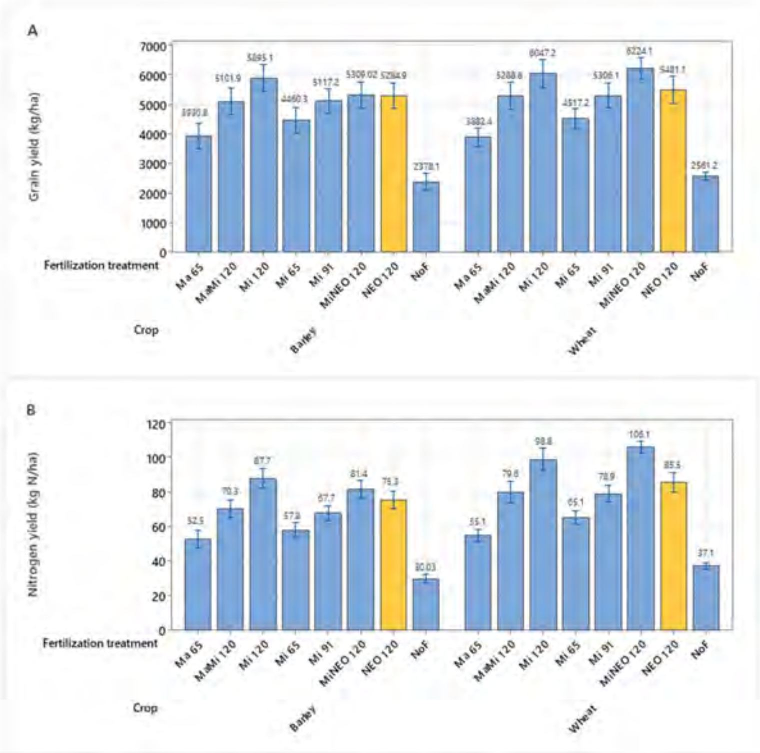


Figure 3. (**A**): Grain yield (15% water content), and (**B**) nitrogen yield, in kg N ha^{-1} ± standard error of the means. Results from Series 1 in 2021 and 2022, with three trials in barley (**left**) and three trials in spring wheat (**right**). The NEO 120 kg N ha^{-1} treatment stands out from other treatments with its distinct yellow coloration.

Regarding wheat grain yield (Figure 4A), MaMi120 yielded significantly higher (451 kg ha^{-1}) than NEO120. On the other hand, NEO120 yielded similar to Mi80 but significantly higher (695 kg ha^{-1}) than ma65.

A similar trend in barley and wheat grain yield was observed for nitrogen yield (Figure 4B). However, the differences between treatments were more pronounced, indicating increased variation in nitrogen yield.

3.4. Nitrogen Effects: Results from All 10 Trials in Series One and Two in 2021 and 2022

The Y axis in Figure 5 represents the nitrogen effect obtained from the range of mineral fertilizers. NEO120 exhibited an equivalent effect on grain yield as 95 kg N ha^{-1} in mineral fertilizer and the same effect on nitrogen yield as 100 kg N ha^{-1} in mineral fertilizer. In simpler terms, 95 kg N ha^{-1} in mineral fertilizer can be substituted with 120 kg N ha^{-1} in NEO when considering wheat and barley grain yield. Similarly, when assessing nitrogen yield, 100 kg N ha^{-1} in mineral fertilizer can be replaced by 120 kg N ha^{-1} in NEO.

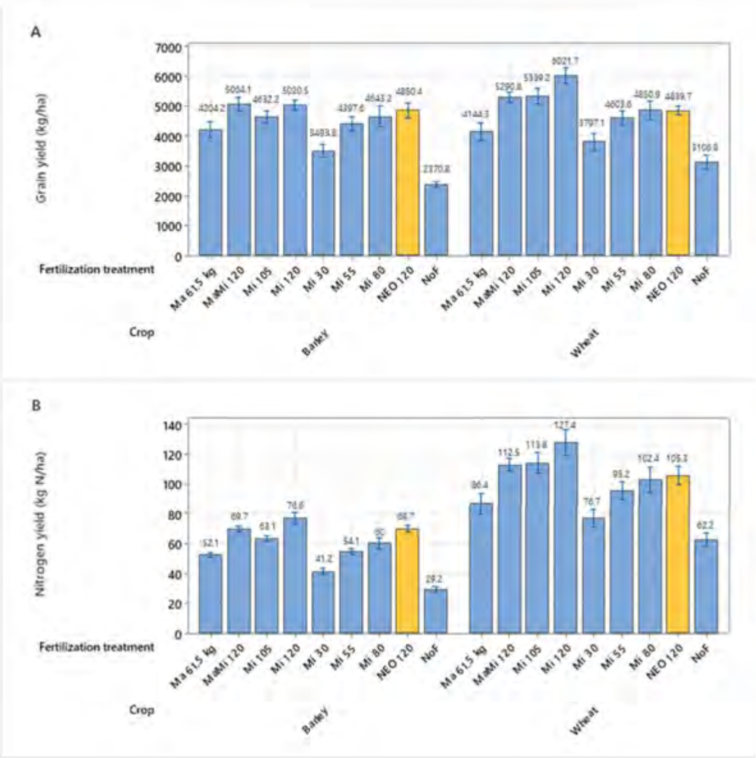


Figure 4. (**A**): Grain yield (15% water content), and (**B**) nitrogen yield, in kg N ha^{-1} ± standard error of the means. Results from Series 2 in 2021 and 2022, with two trials in barley (**left**) and three trials in spring wheat (**right**). The NEO 120 kg N ha^{-1} treatment stands out from other treatments with its distinct yellow coloration.

Ma65 demonstrated a grain yield effect comparable to 50 kg N ha^{-1} in mineral fertilizer, but the nitrogen yield from Ma65 was slightly lower. MaMi120 also exhibited the same grain yield nitrogen effect as 95 kg N ha^{-1} in mineral fertilizer. However, the nitrogen yield from MaMi120 was slightly lower in comparison.

3.5. Sum up All Average Yields

The following table, Table 5, presents the average barley and wheat grain yields from the most noteworthy treatments in both series one and two in 2021 and 2022 (columns two and three) and series one only (columns four and five).

When considering the combined results from all trials in barley and wheat (columns two and three), MaMi120 yielded 5083 kg ha^{-1} barley grain and 5290 kg ha^{-1} wheat grain. These results were equivalent to the barley yield obtained from NEO120, while in wheat, MaMi120 outperformed NEO120 by 135 kg ha^{-1}. MaMi120 and NEO120 yielded more than 1000 kg ha^{-1} compared to Ma65, with the largest increase observed in wheat. Mi120 yielded 425 kg ha^{-1} higher barley grain and 968 kg ha^{-1} higher wheat grain than NEO120.

Focusing on Series 1 alone (columns three and four), similar yield differences were observed as in the combined results. Additionally, we included the MiNEO120 treatment in this analysis. Regarding barley, MiNEO120 yielded similar to NEO120, while in wheat, it outperformed NEO120 by 743 kg ha^{-1} and MaMi120 by 935 kg ha^{-1}. Remarkably, MiNEO120 even surpassed the wheat grain yield of Mi120.

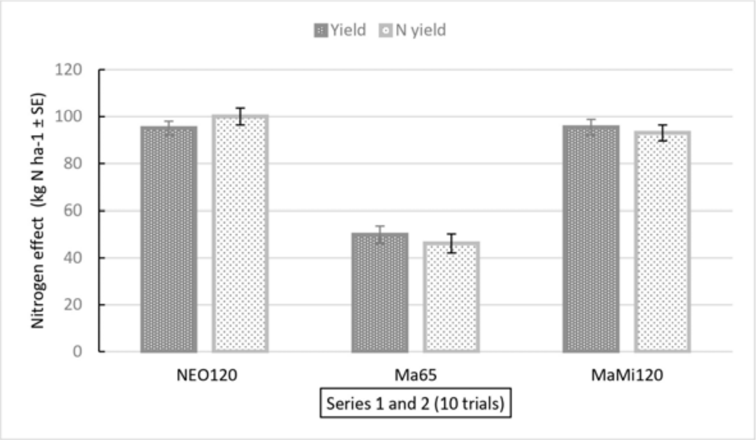


Figure 5. Nitrogen effect of 120 kg N ha^{-1} in NEO (NEO120) compared to the nitrogen effect of 65 kg N ha^{-1} in manure (Ma65) and 120 kg N ha^{-1} in manure with mineral fertilizer (MaMi120). Results are based on data from ten trials in Series 1 and 2 combined. Results are provided for grain yield data and N yield (bars with light pattern).

Table 5. The average yields per ha of the Ma65, MaMi120, NEO120, and Mi120 treatments in all 5 trials in barley and 5 trials in wheat in 2021 and 2022 (Series 1 and 2 combined). Columns four and five give the same only from the trials in Series 1 from the same years, also containing the average yield effects from the MiNEO120 treatment.

Fertilization Treatment	Average Yield (kg ha^{-1}) All Trials 2021 and 2022 Barley (5 Trials)	Average Yield (kg ha^{-1}) All Trials 2021 and 2022 Wheat (5 Trials)	Average Yield (kg ha^{-1}) Series 1 2021 and 2022 Barley (3 Trials)	Average Yield (kg ha^{-1}) Series 1 2021 and 2022 Wheat (3 Trials)
Ma65	4068	4013	3931	3882
MaMi120	5083	5290	5102	5289
NEO120	5068	5155	5285	5481
MiNEO120	-	-	5309	6224
Mi120	5443	6123	5895	6047

4. Discussion

4.1. Nitrogen Fertilization Effect of NEO

The present studies aimed to determine the comparative variances in yield and fertilizer efficacy among NEO, mineral fertilizers, cattle slurry, and other farmers' alternatives. The experiments were located in representative parts of Norway's most crucial grain production areas. The intended nitrogen (N) application level for the study was set at 120 kg N ha^{-1}, which aligns with the average N level commonly used for barley and is slightly lower (around 1–2 kg) than the average N level employed for wheat in practical farming within the region. The yields obtained from applying 120 kg N ha^{-1} using mineral fertilizer fell within the same range as those observed in the official Norwegian variety trials. [24].

During the initial two trials conducted in 2020, we observed a substantial increase in wheat yield when utilizing filtered slurry compared to the untreated slurry, with a difference of 756 kg ha^{-1}. Similarly, in the case of barley, the filtered slurry resulted in a yield increase of 447 kg ha^{-1} compared to the untreated slurry. These findings indicate that by simply filtering the slurry, the fertilizer effect was enhanced by approximately 10–14 percent in barley and wheat, respectively. It is worth noting that despite the reduced volume and applied nitrogen amount due to filtration, the positive impact on yield was still significant. This phenomenon can be attributed to the reduced carbon-to-nitrogen (C/N) ratio observed in the filtered slurry, resulting in an enhanced nitrogen mineralization process [25,26]. This can elucidate a portion of the positive yield effect achieved by implementing the N2 Applied technology.

Our study focused on assessing the potential of NEO as a substitute for nitrogen (N) from mineral fertilizers. The results revealed that applying 120 kg N ha^{-1} in NEO resulted in a similar grain yield as using 95 kg N ha^{-1} in mineral fertilizer, which is a 20% reduction in yield compared to the 120 kg N ha^{-1} in mineral fertilizer. However, NEO exhibited slightly better performance when considering nitrogen yield, with 120 kg N-min ha^{-1} in NEO yielding comparable results to 100 kg N ha^{-1} in mineral fertilizer (a 16.7% reduction) [27–29].

We also examined the impact of combining untreated manure with mineral fertilizers on grain yield. Notably, applying 65 kg N ha^{-1} in untreated manure supplemented with 55 kg N ha^{-1} in mineral fertilizer yielded the same as using 95 kg N ha^{-1} in mineral fertilizer and 120 kg N ha^{-1} in NEO for both barley and wheat grain yield. Interestingly, within 120 kg N ha^{-1} in NEO, 60 kg N is added to the manure through plasma treatment. These findings indicate that NEO may suitably replace the combination of untreated manure with mineral fertilizers, which can be an effective strategy to reduce reliance on mineral fertilizers while maintaining comparable yields.

To further analyze the results, we examined the average wheat and barley yields, as presented in Figures 2–4 and Table 5. Our grain trials set the targeted nitrogen value at 120 kg N ha^{-1}. To achieve this level using NEO, we applied 37.5 tons ha^{-1} and 34 tons ha^{-1} in 2021 and 2022, respectively, considering the varying nitrogen content in NEO. Correspondingly, the amounts of untreated slurry applied were 41 tons ha^{-1} and 37 tons ha^{-1}, resulting in an average of 39 tons ha^{-1} of untreated slurry. Using this information, we calculated the yields obtained from different combinations of 39 tons ha^{-1} of cattle slurry and mineral fertilizer:

When applying 39 tons ha^{-1} of untreated cattle slurry (Ma65), we harvested a barley yield of 4068 kg ha^{-1} and a wheat yield of 4013 kg ha^{-1}. By combining 39 tons ha^{-1} of untreated slurry with mineral fertilizers (Opti-NS) up to 120 kg N ha^{-1} (MaMi120), we observed improved yields, with barley reaching 5083 kg ha^{-1} and wheat reaching 5290 kg ha^{-1}.

To explore the potential of alternative fertilization techniques, we filtered 39 tons of untreated slurry. Then, we processed it through the N2 Applied unit, resulting in 35 tons of NEO with a nitrogen content of 120 kg N. Applying 35 tons ha^{-1} of NEO (NEO120) yielded a barley yield of 5068 kg ha^{-1} and a wheat yield of 5155 kg ha^{-1}, the same level as the combination of cattle slurry and mineral fertilizer up to 120 kg N ha^{-1}.

Furthermore, we examined the effects of solely using mineral fertilizers with a nitrogen content of 120 kg N ha^{-1}, specifically Yara 18-3-15 (Mi120). This approach resulted in even higher yields, with barley reaching 5443 kg ha^{-1} and wheat reaching 6123 kg ha^{-1}.

4.2. NEO at Three Leaf Stage

Interestingly, throughout our trials, we consistently observed no evidence of damage to barley and wheat plants when NEO was applied during the three-leaf stage. This finding indicates that applying NEO at this particular growth stage does not result in any discernible harm to the crops.

The treatment known as MiNEO120 involved the application of NEO (108 kg N ha^{-1}) at the three-leaf stage, while a small quantity (12 kg N ha^{-1}) of mineral fertilizer was applied on the sowing day. This approach yielded a crop production increase of 743 kg ha^{-1} compared to the sole application of NEO (NEO120) at sowing, based on six trials conducted in 2021 and 2022. Additionally, the MiNEO120 treatment demonstrated slightly higher wheat yields than the application of 120 kg N ha^{-1} as mineral fertilizer on the sowing day (Mi120). However, when it came to barley, no significant yield improvement was observed with the MiNEO120 treatment compared to the NEO120 treatment. In fact, the MiNEO120 treatment decreased yield by 586 kg ha^{-1} when compared to the Mi120 treatment.

In contrast, our trials conducted in 2020 using the MiNEO treatment with an N application rate of 104 kg N ha^{-1} resulted in significantly lower yields. Specifically, there was a decrease of 469 kg ha^{-1} compared to using the NEO102 treatment in wheat. However, in the case of barley, both treatments yielded approximately the same results in 2020. These findings highlight the diverse effects that different nitrogen application methods and rates can have on wheat and barley yields.

However, the high yield observed in the MiNEO120 treatment can be partially attributed to trial number three out of the six trials conducted in series one during 2021 and 2022. MiNEO120 yielded a notably higher yield in this particular trial than MiNEO120 in the remaining five trials. We have thoroughly analyzed the weather conditions in the weeks following sowing and the application of NEO in the MiNEO treatment. However, our investigation did not yield any definitive explanations for this discrepancy.

On the other hand, it is widely acknowledged that wheat has a later nitrogen uptake during the growing season compared to barley [30]. This difference in nitrogen absorption timing may help explain why MiNEO120 consistently resulted in higher wheat yields than barley in most of our trials. Another contributing factor could be the high nitrification potential of NEO, which leads to greater availability of plant-accessible nitrate over a concentrated period of 3–4 days following application [18]. This rapid release of nitrate may particularly benefit wheat [31,32].

It is important to note that all other fertilizer treatments, apart from MiNEO120, were applied solely on the sowing day. Therefore, our experiments do not provide insights into how these alternative treatments would have performed if they had been applied partially on the sowing day and partially at the three-leaf stage, similar to the MiNEO120 treatment. Consequently, the only valid comparison is between MiNEO120 and NEO120.

4.3. Limitations and Further Research

The ammonia and nitrate levels in NEO and the mineral fertilizer used in our experiments are similar. However, despite this similarity, it is astonishing that the crop yield obtained from 120 kg N ha^{-1} in NEO is equivalent to the yield achieved from just 95 kg N ha^{-1} in the mineral fertilizer. Furthermore, the yield obtained from 120 kg N ha^{-1} in NEO is the same as that obtained from the combination of untreated slurry and mineral fertilizer Opti-NS at the same nitrogen application rate. However, The nitrogen yields obtained from NEO were slightly superior, delivering an equivalent nitrogen effect as that of 100 kg N ha^{-1} in mineral fertilizer. This suggests that approximately 20 kg ha^{-1}, corresponding to 17% of the plant-available nitrogen in NEO, is lost through other means.

Regrettably, we cannot provide a conclusive explanation for these unexpected outcomes. Notably, the low pH in NEO should typically mitigate the majority of ammonia leakage [33]. Nevertheless, one possibility to consider is that the high soil nitrification potential in the initial days following the application of NEO could lead to nitrate losses without adequate plant absorption. Additionally, there is a risk that nitrogen could be lost as nitrous oxide through denitrification, further exacerbating the situation. Therefore, additional research investigating the emissions or leaching potential of NEO is necessary to gain a clearer understanding of this phenomenon.

5. Conclusions

The current study aimed to investigate and compare the effects of Nitrogen Enriched Organic fertilizer (NEO) made from cattle slurry on barley and wheat grain yields to other conventional fertilizers used in agriculture. The results indicated that 120 kg N ha^{-1} in NEO yielded in the same range of cereal grains as 95 kg N ha^{-1} in mineral fertilizer. Moreover, the combination of untreated slurry and 55 kg N ha^{-1} in mineral fertilizer Opti-NS yielded the same as 120 kg N ha^{-1} in NEO. Surprisingly, the combination of 12 kg N ha^{-1} in mineral fertilizer applied at sowing, alongside 108 kg N ha^{-1} in NEO administered at the three-leaf stage, resulted in higher wheat yields compared to the application of 120 kg N ha^{-1} of NEO solely spread at sowing in two out of three experimental years. Additionally, the direct application of NEO onto the plants exhibited no observable signs of harm. Lastly, it is worth noting that filtering the slurry yielded higher cereal grain yields compared to using the untreated slurry. Thus, while NEO and mineral fertilizers have similar N content, utilizing NEO resulted in a cereal grain yield 15–20% lower than that achieved with mineral fertilizer. However, it still yielded 20–30% higher than the native amount of cattle slurry it originated. Nevertheless, it is worth noting that approximately 17% of the nitrogen in NEO appears to be lost through unidentified means without contributing to grain or nitrogen yields.

Author Contributions: Conceptualization, T.C. and S.Ø.S.; methodology, T.C. and S.Ø.S.; validation, T.C. and S.Ø.S.; formal analysis, H.M.; investigation, T.C.; data curation, S.Ø.S. and H.M.; writing—original draft preparation, T.C., S.Ø.S. and H.M.; writing—review and editing, T.C., S.Ø.S. and H.M.; visualization, T.C., S.Ø.S. and H.M.; supervision, S.Ø.S.; project administration, T.C.; funding acquisition, T.C. and S.Ø.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Research Council of Norway (grant number 309640 Plasmabehandlet husdyrgjødsel—gjødselvirkning, miljøpåvirkning og klimagassutslipp) and the Company N2 Applied.

Data Availability Statement: The data is available upon request.

Acknowledgments: The authors would like to thank the staff at NLR Trøndelag, NLR Innlandet, NLR Østafjells, and NLR Viken for their help in conducting the field trials. We also thank Morten Tofastrud and Elisabeth Røe at Inland Norway University of Applied Sciences for their support in project administration. In addition, we would like to acknowledge the project's funders.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. *The Future of Food and Agriculture, Trends and Challenges*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2017; ISBN 978-92-5-109551-5.
2. Intergovernmental Panel on Climate Change. *Climate Change and Land: IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes in Terrestrial Ecosystems*; Cambridge University Press: Cambridge, UK, 2022.
3. Vermeir, I.; Verbeke, W. Sustainable Food Consumption: Exploring the Consumer "Attitude–Behavioral Intention" Gap. *J. Agric. Environ. Ethics* **2006**, *19*, 169–194. [CrossRef]
4. Augustin, M.A.; Riley, M.; Stockmann, R.; Bennett, L.; Kahl, A.; Lockett, T.; Osmond, M.; Sanguansri, P.; Stonehouse, W.; Zajac, I.; et al. Role of food processing in food and nutrition security. *Trends Food Sci. Technol.* **2016**, *56*, 115–125. [CrossRef]
5. Janker, J.; Mann, S.; Rist, S. Social sustainability in agriculture—A system-based framework. *J. Rural Stud.* **2019**, *65*, 32–42. [CrossRef]
6. Rajic, S.; Đorđević, V.; Tomasevic, I.; Djekic, I. The role of food systems in achieving the sustainable development goals: Environmental perspective. *Bus. Strategy Environ.* **2022**, *31*, 988–1001. [CrossRef]
7. Dong, N.-Q.; Lin, H.-X. Higher yield with less nitrogen fertilizer. *Nat. Plants* **2020**, *6*, 1078–1079. [CrossRef] [PubMed]
8. Houlton, B.Z.; Almaraz, M.; Aneja, V.; Austin, A.T.; Bai, E.; Cassman, K.G.; Compton, J.E.; Davidson, E.A.; Erisman, J.W.; Galloway, J.N.; et al. A World of Cobenefits: Solving the Global Nitrogen Challenge. *Earth's Future* **2019**, *7*, 865–872. [CrossRef] [PubMed]
9. Bondada, B.R.; Oosterhuis, D.M. Canopy photosynthesis, specific leaf weight, and yield components of cotton under varying nitrogen supply. *J. Plant Nutr.* **2001**, *24*, 469–477. [CrossRef]
10. Rütting, T.; Aronsson, H.; Delin, S. Efficient use of nitrogen in agriculture. *Nutr. Cycl. Agroecosyst.* **2018**, *110*, 1–5. [CrossRef]
11. FAO. *World Fertilizer Trends and Outlook to 2022*; FAO: Rome, Italy, 2019.
12. Upendra, M.S.; Rajan, G.; Gautam, P.P. Nitrogen Fertilization I: Impact on Crop, Soil, and Environment. In *Nitrogen Fixation*; Everlon Cid, R., Ademar Pereira, S., Eds.; IntechOpen: Rijeka, Croatia, 2019; Chapter 5.
13. European Commission. Sustainable Use of Nutrients: The Common Agricultural Policy Supports Farmers in the Safe and Efficient Use of Nutrients. Available online: https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability/low-input-farming/nutrients_en (accessed on 18 July 2022).

14. Graves, D.B.; Bakken, L.B.; Jensen, M.B.; Ingels, R. Plasma Activated Organic Fertilizer. *Plasma Chem. Plasma Process.* **2019**, *39*, 1–19. [CrossRef]
15. Ingels, R.; Graves, D.B. Improving the Efficiency of Organic Fertilizer and Nitrogen Use via Air Plasma and Distributed Renewable Energy. *Plasma Med.* **2015**, *5*, 257–270. [CrossRef]
16. N2 Applied. Nitrogen Enriched Organic Fertiliser. Available online: https://n2applied.com/ (accessed on 19 July 2022).
17. Mousavi, H.; Cottis, T.; Pommeresche, R.; Dörsch, P.; Solberg, S.Ø. Plasma-Treated Nitrogen-Enriched Manure Does Not Impose Adverse Effects on Soil Fauna Feeding Activity or Springtails and Earthworms Abundance. *Agronomy* **2022**, *12*, 2314. [CrossRef]
18. Mousavi, H.; Solberg, S.Ø.; Cottis, T.; Dörsch, P. Nitrogen-Enriched Organic Fertilizer (NEO) Elevates Nitrification Rates Shortly after Application But Has No Lasting Effect on Nitrification in Agricultural Soils. Preprint. 2023. Available online: https://doi.org/10.21203/rs.3.rs-2565156/v1 (accessed on 10 March 2023).
19. Mousavi, H.; Cottis, T.; Hoff, G.; Solberg, S.Ø. Nitrogen Enriched Organic Fertilizer (NEO) and Its Effect on Ryegrass Yield and Soil Fauna Feeding Activity under Controlled Conditions. *Sustainability* **2022**, *14*, 2005. [CrossRef]
20. Yara. YaraMila™. Available online: https://www.yara.com/crop-nutrition/products-and-solutions/global-fertilizer-brands/yaramila/ (accessed on 20 July 2022).
21. Yara International. Yara Opti NS. Available online: https://www.yara.no/gjoedsel/produkter/yarabela/yarabela-opti-ns-27-0-0-4s/ (accessed on 3 May 2023).
22. Zadoks, J.C.; Chang, T.T.; Konzak, C.F. A decimal code for the growth stages of cereals. *Weed Res.* **1974**, *14*, 415–421. [CrossRef]
23. yr.no. Norwegian Meteorological Institute and the Norwegian Broadcasting Corporation; Weather Data History. 2023. Available online: www.yr.no (accessed on 11 March 2023).
24. Strand, E. *Jord-Og Plantekultur 2021 Forsøk i Korn, Olje-Og Belgvekster, Engfrøavl Og Potet 2020*; NIBIO: Amsterdam, The Netherlands, 2021. Available online: https://nibio.brage.unit.no/nibio-xmlui/handle/11250/2740694 (accessed on 11 March 2023).
25. Watson, C.A.; Atkinson, D.; Gosling, P.; Jackson, L.R.; Rayns, F.W. Managing soil fertility in organic farming systems. *Soil Use Manag.* **2002**, *18*, 239–247. [CrossRef]
26. Biswas, D.; Micallef, S.A. *Safety and Practice for Organic Food*; Academic Press: Cambridge, MA, USA, 2019.
27. Sutton, M.; Howard, C.; Mason, K.; Brownlie, W.; Cordovil, C. Nitrogen Opportunities for Agriculture, Food & Environment. UNECE Guidance Document on Integrated Sustainable Nitrogen Management. 2022. Available online: https://nora.nerc.ac.uk/id/eprint/534033/ (accessed on 10 March 2023).
28. Jackson, D.R.; Smith, K.A. Animal manure slurries as a source of nitrogen for cereals; effect of application time on efficiency. *Soil Use Manag.* **1997**, *13*, 75–81. [CrossRef]
29. Ladha, J.K.; Pathak, H.J.; Krupnik, T.; Six, J.; van Kessel, C. Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects. In *Advances in Agronomy*; Academic Press: Cambridge, MA, USA, 2005; Volume 87, pp. 85–156.
30. Bosshard, C.; Sørensen, P.; Frossard, E.; Dubois, D.; Mäder, P.; Nanzer, S.; Oberson, A. Nitrogen use efficiency of 15N-labelled sheep manure and mineral fertiliser applied to microplots in long-term organic and conventional cropping systems. *Nutr. Cycl. Agroecosyst.* **2009**, *83*, 271–287. [CrossRef]
31. Alcoz, M.M.; Hons, F.M.; Haby, V.A. Nitrogen Fertilization Timing Effect on Wheat Production, Nitrogen Uptake Efficiency, and Residual Soil Nitrogen. *Agron. J.* **1993**, *85*, 1198–1203. [CrossRef]
32. Cox, W.J.; Reisenauer, H.M. Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. *Plant Soil* **1973**, *38*, 363–380. [CrossRef]
33. Fangueiro, D.; Hjorth, M.; Gioelli, F. Acidification of animal slurry—A review. *J. Environ. Manag.* **2015**, *149*, 46–56. [CrossRef] [PubMed]

Inland Norway
University of
Applied Sciences

In a time of rapid human population growth, rising food prices, limited arable land, and environmental farmland degradation, there is a growing demand for eco-friendly fertilizer solutions. Nitrogen Enriched Organic fertilizer (NEO), an innovative product, is produced using a method that captures nitrogen from the air and blends it with bio-based fertilizers. Our research assessed NEO's impact on soil health and crop yields. We observed that NEO, alongside other fertilizers, had no adverse effects on soil fauna feeding activity, earthworms, springtails, or nitrification. While NEO initially increased nitrification rates in controlled conditions, this effect did not persist after six months in the field. Regarding crop yields, NEO produced slightly lower yields than mineral fertilizers in controlled and field settings but outperformed cattle slurry, delivering 20–30% higher grain yields. In conclusion, NEO can contribute to the journey towards sustainable global food production systems.