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

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In the face of population growth, rising food production costs, limited arable land availability, and environmental degradation of farmlands, adopting innovative technologies, particularly those related to organic waste recycling and nutrient recovery, has emerged as an imperative strategy. These technologies are crucial in bolstering 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 slurries or digestates 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 73-hour soil 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. Interestingly, 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 nitrification.

Keywords: environment, innovative fertilizers, nitrogen, manure, sustainability, resilience, waste management

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 (UN 2017). At the same time, 12 million hectares of arable land are lost annually (UN 2019), which means that productivity has to increase in the remaining area. This also means that agricultural systems have become increasingly dependent on mineral nitrogen (N) fertilizers (Matson et al. 1997, Mancus 2007, Lu and Tian 2017) .

Nitrogen is a critical nutrient for promoting plant growth; however, it is also one of the primary limiting nutrients in agroecosystems (Dong and Lin 2020). Over the past six decades, agricultural productivity has significantly increased, tripling its output (FAO 2017), while global N inputs into agriculture have grown remarkably, reaching an eightfold increase (FAO 2022).

The 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 underscores the significance of embracing a circular economy approach and emphasizes the importance of expanding our utilization of renewable and biobased resources, such as recycling nutrients from biowastes in agroecosystems.

Although increased use of N fertilizers boosts crop productivity (Harris et al. 1996, Abraha et al. 2015, Cinar et al. 2020), it generates severe drawbacks (Upendra et al. 2019). In addition to increasing fertilization expenses, more

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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 (Mulvaney et al. 2009, McAllister et al. 2012, Lassaletta et al. 2014, Chen et al. 2020). 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 (UN 2020).

Moreover, from a soil perspective, altering nutrients and causing imbalances with external additives lead to changes in functional microbial communities (Savci 2012, Bell et al. 2015, Lu and Tian 2017) 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 (EC 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" (UN 2020, EC 2022). The latter targets 20% less fertilizer consumption and a minimum 50% decrease in nutrient leaching by 2030.

In this context, a practical approach involves the development of sustainable, organic-based agricultural amendments, such as nutrient recycling from animal slurries or anaerobic digestion byproducts. However, utilizing digestates and slurries as a considerable nutrient source is confronted by several critical limitations. These constraints encompass nutrient concentration, contaminants, odorous components, and environmental management concerns (Mickan et al. 2022, O'Connor et al. 2022).

Aiming to alleviate the abovementioned concerns and drive toward a more sustainable future, N2 Applied (Asker, Norway) has developed a unit for enriching organic amendments such as slurries and digestates with atmospheric N. The final product is an N-rich, acidified, biobased fertilizer termed "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 N is then brought into contact with water, forming nitrous acid (HNO_2) and nitric acid (HNO_3), which are added to the slurries or digestates. This process lowers the pH of the mixture, which may impact soil microbial activity (N2 Applied 2022). Furthermore, slurries treated with NEO undergo filtration and transformation, resulting in a more liquefied state devoid of unpleasant odors. This enhanced liquefaction facilitates a more precise application of NEO-treated slurries to the field (Ingels and Graves 2016, Graves et al. 2019, N2 Applied 2022). The compact size of the N2 application device also enables decentralized fertilizer production for farmers, boosting the self-sufficiency of stakeholders (N2 Applied 2022).

The NEO technology is novel and untested in the existing literature, in contrast to other post-treatment methods currently in use or under development, e.g., ammonia stripping, algae cultivation, biochar application, and hydrochar production (O'Connor et al. 2022). Given NEO's novelty as a product, it is essential to thoroughly assess its potential adverse effects on soil-dwelling organisms, ecological communities, and soil nutrient cycling before contemplating its introduction 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 (Prosser and Nicol 2012, Creamer et al. 2022, 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 (Griffiths et al. 2016, Bünemann et al. 2018, 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) (Killham 1998, Purkhold et al. 2000, Kartal et al. 2012). 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 because it regulates 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 and 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 plants (Drury 2007), nitrate leaching and gaseous nitrous oxide (N_2O) formation also increase, decreasing the N retention and plant availability of N (Fowler et al. 2013, Beeckman et al. 2018). Hence, the reduction of nitrification becomes desirable when 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 our prior studies, we determined that NEO slurry not only enhanced crop yields compared to the original feedstock from which it was derived (Cottis et al. 2023) but also exhibited no detrimental impact on soil fauna feeding activity—an integral factor in soil nutrient cycling (Mousavi et al. 2022a, Mousavi et al. 2022b)—or the abundance of springtails and earthworms (soil faunal communities) (Mousavi et al. 2022b).

In this study, we assessed the nitrification potential in two field trials, one grass and the other cereal, 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 22 April, 2020, and 27 April, 2021. In contrast, the grass plots received fertilizer application twice yearly – first in early spring (27 April 2020, and 4 May 2021) and then after the first harvest (24 June 2020, and 15 June 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 the 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% 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 approximately 10% clay, with a pH of 5.7 and 5.1% 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). However, 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, 2022), organic fertilizer (the same untreated cattle slurry used to produce NEO), and no fertilizer (control).

The 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. In 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 administered in liquid form. All fertilizer stocks were thoroughly swirled before bottling to ensure a uniform distribution of particles in the liquid. 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 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 approximately 20% more precipitation than usual. Consequently, the cereal yield in 2021 was above average, likely influenced by 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 are 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, ≈ six months after fertilization, soil sampling was conducted at both field locations. On the sampling day, temperatures hovered at approximately 5–6 °C following a period of rainfall 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 being transferred 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, it was 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 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 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 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 (Killham 1990, Wang 2010, Thion and Prosser 2014). The nitrite + nitrate (NO_2^- + NO_3^-) concentration was determined following the assay adapted from Doane and 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 soil samples were estimated from the NO_2^- + NO_3^- accumulation 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 (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 the concentrations of NO_2^- and NO_2^- + NO_3^- as described above.

Considering that nitrification is pH-sensitive and that the rate declines at low pH values (Ste-Marie and 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 similar to the procedures for field-fertilized soil samples, 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 extractions. As in the experiment with field-fertilized soil, all samples were screened for soil pH change before and after incubation.

Lab-fertilized soil loosely placed

Over time, NO_2^- + NO_3^- accumulation was measured by sacrificing three tubes per treatment at 1, 25, and 73 h into the incubation. NO_2^- + NO_3^- was extracted by adding 30 ml of 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 field-fertilized soil, and Lab-fertilized soil incubated as agitated soil slurry sections.

Data handling and statistical analyses

The spectrophotometry data regarding NO_2^- + NO_3^- accumulation were 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 nitrite accumulation was negligible, nitrification potentials were calculated from the (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 the data. The graphs were generated using the 'ggplot2' package (Wickham 2016) and the 'stat_summary' function within R-Studio version 2023.06.2 Build 561 (copyrighted © 2009–2023 by Posit Software, PBC). The error bars in these plots were computed based on standard errors.

Results

Field-fertilized soil

The nitrification potentials observed in soil samples, collected approximately six months after various fertilization treatments in the field, revealed that fertilization had no discernible effect on 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 nonsignificant (NO_2^- + NO_3^-)-N accumulation rate (32.8 µg g^{-1} dry weight soil day^{-1}) than soils that had received mineral fertilizer (31.4 µg g^{-1} DW soil day^{-1}), organic fertilizer (31.3 µg g^{-1} DW soil day^{-1}), and NEO (31.1 µg g^{-1} DW soil day^{-1}) (Fig.1A, Figs. S1A–D to S4A–D, Table S1). In the grassland experiment, organically fertilized soil had a slightly higher nitrification rate (19.2 µg g^{-1} DW soil day^{-1}) than soil that had received mineral fertilizer (18.1 µg g^{-1} DW soil day^{-1}), no fertilizer (17.4 µg g^{-1} DW soil day^{-1}), or NEO (15.9 µg g^{-1} DW soil day^{-1}) (Fig. 1B, Figs. 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 than in the cereal field (Figs.1AB, Table S1).

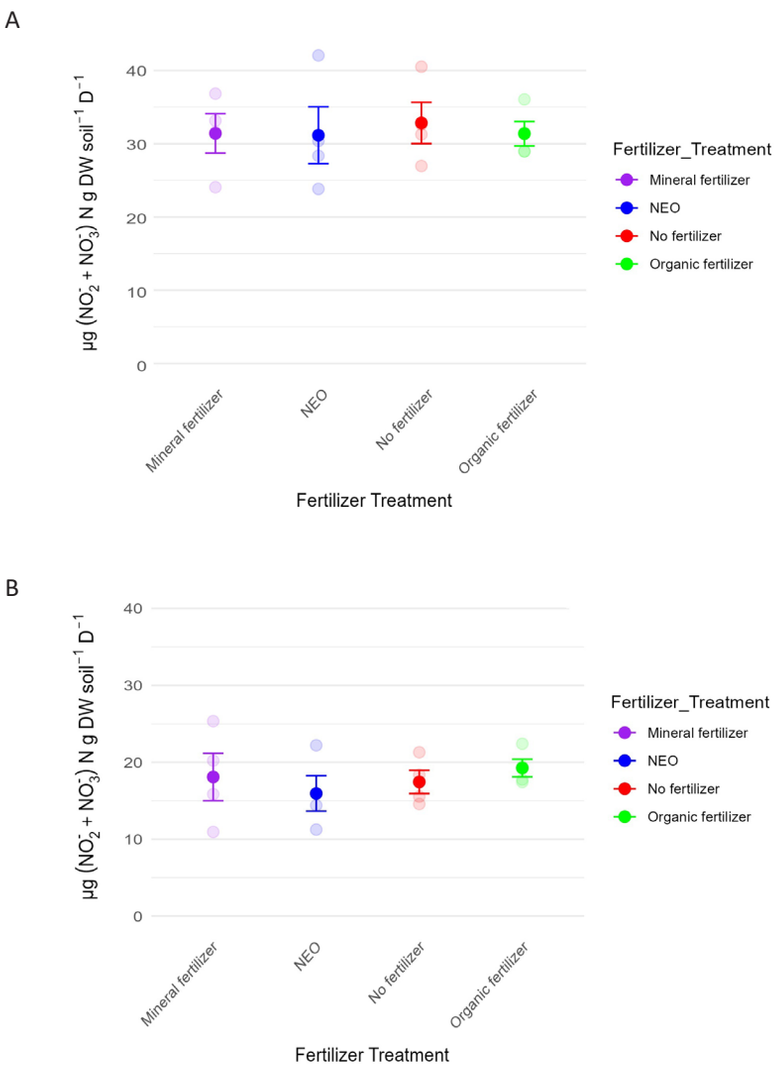

Fig. 1 The effect of different fertilization treatments on nitrification potentials in the cereal (A) and grass field (B) soils collected from the fertilized fields ≈ six months after fertilization and incubated as agitated soil slurries for 66 hours. NEO = nitrogen-enriched cattle slurry, organic fertilizer = untreated cattle slurry. Error bars show standard errors (n=4).

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 and 7.1. The native pH of fertilized grass field soils (n=4) varied between ca. 5.4–5.6. As expected, adding ammonium in the form of different fertilizers decreased the pH during the 66-hour incubation. The grass field samples' pH decreased more than the cereal field samples (Figs. 2AB).

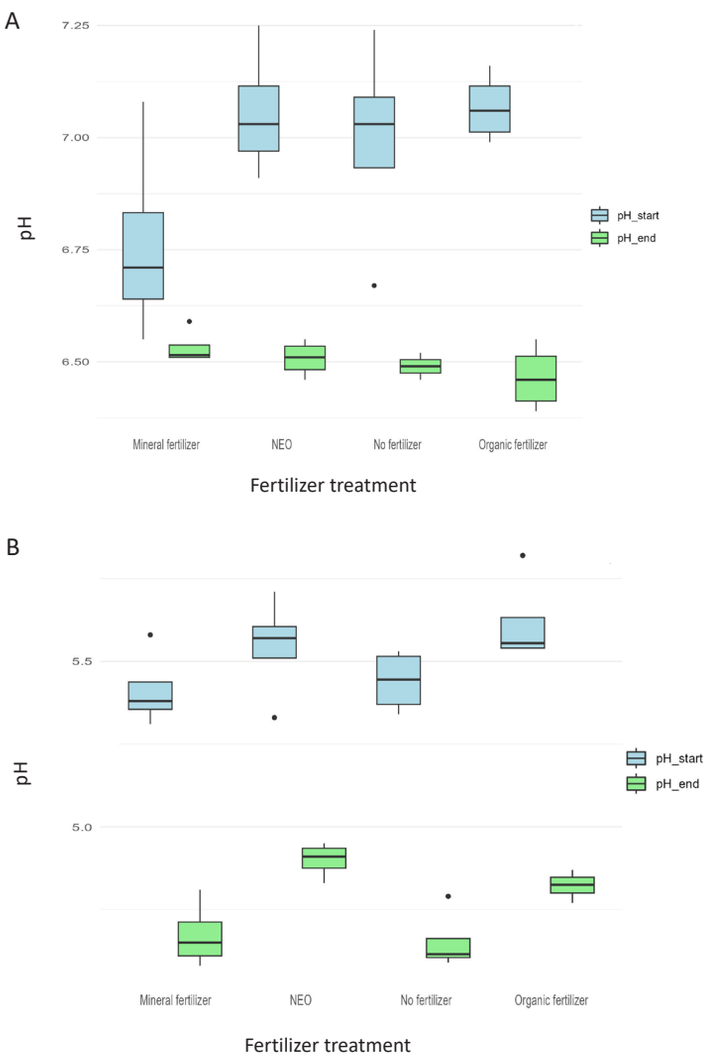

Fig. 2. pH changes before and after a 66-hour incubation as agitated slurries of cereal (A) and grass field (B) soils treated with different fertilizers in the field and sampled ≈ six months after fertilization. NEO = nitrogen-enriched cattle slurry, organic fertilizer = untreated cattle slurry. Error bars show standard errors (n=4).

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, followed by NEO S, indicating a higher (NO_2^- + NO_3^-)-N accumulation rate (257.3 and 108.4 µg g^{-1} DW soil day^{-1}, respectively) than ammonium chloride (54 µg g^{-1} DW soil day^{-1}), untreated biogas digestate (Raw D) (46.5 µg g^{-1} DW soil day^{-1}),

acidified untreated slurry (Raw S acidified) (41.9 µg g^{-1} DW soil day^{-1}), and untreated slurry (Raw S) (34.9 µg g^{-1} DW soil day^{-1}) (Fig.3A, Figs.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^{-1} DW soil day^{-1}, respectively) than ammonium chloride (28.7 µg g^{-1} DW soil day^{-1}), raw D (20.3 µg g^{-1} DW soil day^{-1}), raw S (17.7 µg g^{-1} DW soil day^{-1}), and raw S acidified (12.1 µg g^{-1} DW soil day^{-1}) (Fig. 3B, Figs. 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, similar to the first experiment, nitrification rates were lower in the grass field soil than in the cereal field soil.

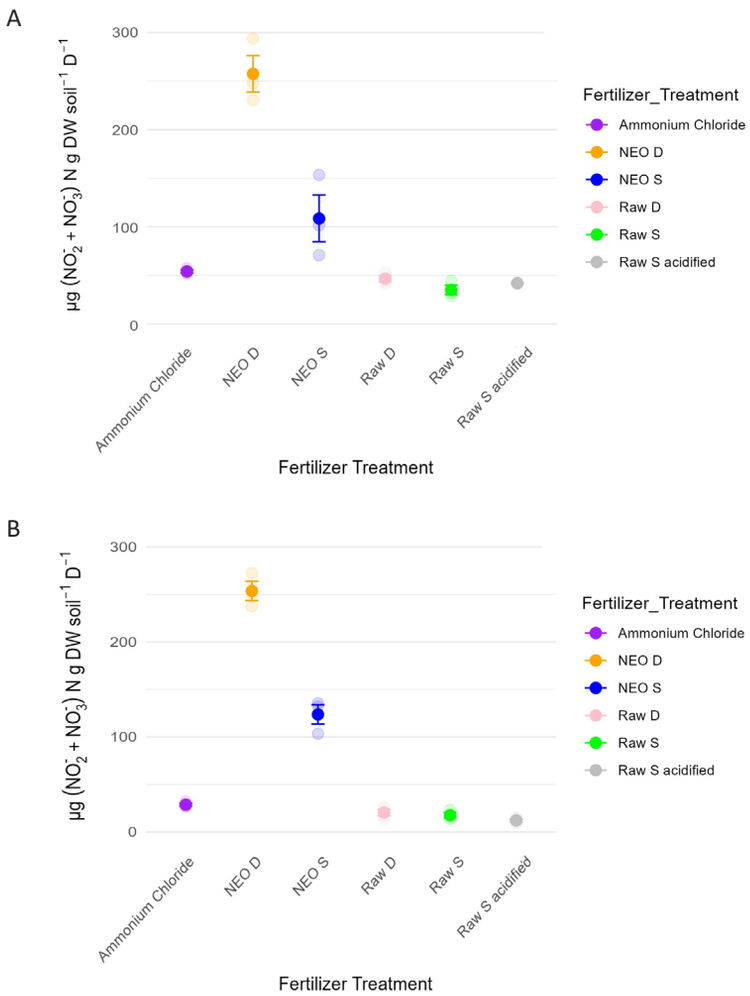

Fig. 3. The effect of lab fertilization with different fertilization treatments on nitrification potentials in the cereal (A) and grass field (B) soils incubated as agitated soil slurries for 43 hours. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries. Raw S = untreated 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 standard errors (n=3).

The pH changed less throughout the 43-hour incubation than in the previous experiment, which used 66 hours. The native average pH of fertilized cereal samples (n=3) varied between ca. 6.7–7.5. Additionally, the native average pH of fertilized grass field soils (n=3) varied between ca. 5.2–6.7 (Figs. 4AB). 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 values to the beginning (Fig. 4A). Nonetheless, within grass field samples, NEO S, NEO D, raw S acidified, and ammonium chloride exhibited a slight increase in average pH (n=3), whereas raw S and raw D had relatively the same pH as the start (Fig. 4B).

Fig. 4. pH changes before and after a 43-hour incubation experiment with agitated soil slurries from the cereal (A) and grass fields (B) amended with different fertilizers in the lab. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries. 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 standard errors (n=3).

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 ($p \leq 0.001$).

Similar to the slurried soil, NEO D showed the most considerable nitrification rate (60.1 µg g^{-1} DW soil day^{-1}), which was significantly higher than that of ammonium chloride (24.5 µg g^{-1} DW soil day^{-1}) and NEO S (20.5 µg g^{-1} DW soil day^{-1}). However, raw D (12.9 µg g^{-1} DW soil day^{-1}) and Raw S (0 µg g^{-1} DW soil day^{-1}) had the lowest average nitrification rates (Fig. 5, Figs.S21A–D to S25A–D, Table S1).

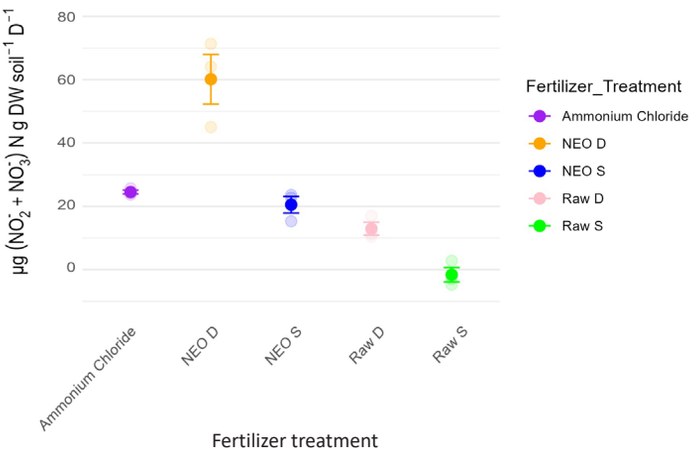

Fig. 5. The effect of lab fertilization with different fertilization treatments on nitrification potential in cereal field soil incubated as non-agitated loose soil for 73 hours. The fertilizer amounts were adjusted to the same NH_4^+ content in the soil slurries. Raw S = untreated cattle slurry, Raw 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 standard errors (n=3)

Discussion

The production of Nitrogen-Enriched Organic fertilizers (NEOs) 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 biobased, its high nitrite content and low pH raise concerns regarding the formation of 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 and Groffmand 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 because it can provide crucial insights into the sustainability and safety of adopting this fertilizer technology. This importance is underscored by the fact that alterations in nitrogen cycling pathways within the soil, prompted by applying biological amendments, can potentially mitigate nitrogen loss. However, this aspect remains a relatively unexplored field in the current research (Shanmugam et al. 2021).

Through our research, we sought to contribute to the knowledge base required for making informed decisions about NEO applicability in agricultural practices, ensuring responsible and environmentally friendly fertilization approaches.

Minimal long-term fertilization effects in the field

During our assessment of nitrification rates in soil samples obtained from fields fertilized during autumn, approximately 5–6 months post-fertilization, we observed no remarkable variations in nitrification potentials across the different fertilization treatments in cereal and grass fields. Notably, the extended period between fertilization and sample collection allowed the removal of added N from the soil through different pathways and the subsequent recuperation from potentially harmful effects. Hence, we may conclude that NEO produced from cattle slurry, which was applied in these trials, does not exhibit a legacy effect on soil nitrification during autumn, even after two consecutive years of application.

Nevertheless, our initial anticipation of discerning notable differences in potential nitrification rates between fertilized and unfertilized soil, as reported in similar studies (Wang et al. 2018, Mohanty et al. 2022, Raglin et al. 2022), did not align with the findings of 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 nonfertilized 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 (Ren et al. 2020). 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 functional redundancy among different taxonomic groups of nitrifiers as a potential reason for the lack of a fertilizer-specific response in field soils (Wu et al. 2011, Gu et al. 2017, Raglin et al. 2022).

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 and 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, when subjected to agitation as soil slurries, the cereal and grass field soils that had received different fertilization treatments did not exhibit any discernible distinctions. Despite the lower pH, NEO made from biogas digestate (NEO D) and cattle slurry (NEO S) stimulated the nitrification rates more than other fertilization treatments in both soils, 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 from 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 (O'Connor et al. 2022, 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. One plausible argument is that the radicals generated through the plasma process may give rise to volatile organic compounds (VOCs). Notably, a previous study demonstrated that certain VOCs can indeed stimulate nitrification (Mohanty et al. 2019).

The fact that we found the same stimulation pattern across treatments in the two different soils indicates a NEO-related factor independent of soil. Another argument for the stimulation by NEO could be that the C/N ratio of NEO is smaller than that of untreated cattle slurry or biogas digestate due to 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. In 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 NEO offers as a biobased fertilizer, this study affirms that NEO does not impede the nitrification process in the soil and may be potentially harmless when employed in agroecosystems. Undeniably, it is necessary to acknowledge that the evaluations of nitrification were executed within the laboratory's optimized conditions, which may deviate from the dynamics of real-world scenarios. While these laboratory assays offer valuable insights into the potential effects of NEO and other fertilizers on nitrification, it is crucial to emphasize that they do not serve as direct evidence of field outcomes.

Furthermore, the study sheds light on the short-term effects of NEO and other fertilizers on nitrification in two subboreal soils, and it does not indicate which physiochemical drivers or changes in the soil microbial communities occur. Therefore, the study emphasizes the need for forthcoming field experiments to explore further NEO application's short- and long-term effects on soil nitrification, mineral N changes, microbial dynamics, taxonomic composition, and other environmental implications under different environmental conditions.

Conclusions

Field and laboratory experiments were conducted to examine and compare the impact of Nitrogen Enriched Organic fertilizers (NEOs) with traditional agricultural fertilizers on potential nitrification rates. The study aimed to assess the effects of newly developed biobased fertilizers—NEOs, derived from cattle slurry and biogas digestate—on soil nitrification. 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 under laboratory-optimized conditions, which may not fully represent real-world dynamics. However, when we examined the field soil samples approximately six months after fertilization with NEO, no significant stimulating effect on nitrification was detected compared to other fertilization treatments. This outcome was consistent across different soil types and crops. These findings indicate that the initial stimulatory effects of NEO on nitrification under controlled laboratory settings 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.

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