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Original article

Shifts in the nitrogen cycle under different fertilizer management practices

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Abstract

Human population is dependent on agricultural production, but these activities pose multiple threats to soil health and indirectly to ecological sustainability. Synthesis of fertilizers proved to be a miraculous solution to enhance soil productivity, but this advancement came with many unseen risks. Long-term research stations that have decades long experiments with fertilizers additions are paramount in better understanding long-term impact of fertilizer use. Our study focused on ammonium and nitrate levels found in soils in an experiment of inorganic nitrogen addition than began in 1975. We further directed our attention to soil mineralization potential, nitrate reductase activities and densities of two major microbial functional groups: ammonifiers and denitrifiers. Our data suggests that ammonium has a stronger tendency of soil buildup than nitrate and that increased levels of inorganic nitrogen species also impacted molecular compartments and processes such as mineralization potential, soil microbiota and enzymatic activities. Another result indicates that analysed soils reached a storage limit for phosphorus which threatens to overburden other ecosystems.

Keywords nitrogen biogeochemistry, soil enzymatic activity, nitrate reductase, soil microbiota, long term fertilizer experiments

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Introduction

The extensive use of fertilizers in agriculture, while essential for enhancing crop yields and ensuring food security, poses significant ecological challenges. One of the primary concerns is nutrient runoff, where excess fertilizers, particularly nitrogen and phosphorus, are washed from agricultural fields into nearby water bodies. This runoff leads to eutrophication, a process that results in the over-enrichment of water bodies with nutrients, causing algal blooms and hypoxic conditions, often referred to as "dead zones," where aquatic life cannot survive [1]. Notable examples include the Gulf of Mexico and Lake Erie, where agricultural runoff has severely impacted aquatic ecosystems [2].

The over-application of fertilizers also contributes to soil degradation. Excessive use of chemical fertilizers can alter soil pH, reduce soil organic matter, and disrupt soil microbial communities [3; 4]. These changes can lead to soil acidification and a decline in soil health, making soils less productive over time and more susceptible to erosion [5]. Soil erosion not only reduces agricultural productivity but also contributes to sedimentation in water bodies, further exacerbating water quality issues.

Fertilizer use is also a significant source of greenhouse gas emissions [6; 7]. Nitrous oxide, a potent greenhouse gas, is released from soils following the application of nitrogen-based fertilizers. This gas has a global warming potential approximately 300 times that of carbon dioxide, making it a critical contributor to climate change [8]. The production and transportation of synthetic fertilizers also involve substantial energy use, primarily from fossil fuels, adding to their carbon footprint [9].

Moreover, the reliance on synthetic fertilizers can lead to a dependency that undermines sustainable agricultural practices. Over time, soils can become less fertile naturally, requiring even more fertilizer inputs to maintain crop yields. This cycle can trap farmers in a pattern of increasing fertilizer use, escalating costs, and diminishing returns, which is particularly challenging for smallholder farmers in developing countries [10].

Human health is also at risk due to fertilizer use. Nitrate contamination of drinking water, resulting from fertilizer runoff, poses significant health risks, including methemoglobinemia or "blue baby syndrome" in infants, and potential links to various cancers. Additionally, the volatilization of ammonia from fertilizers can contribute to the formation of fine particulate matter in the atmosphere, which is associated with respiratory and cardiovascular diseases [11].

Addressing these ecological issues requires a multifaceted approach. Implementing best management practices, such as precision agriculture, can help optimize fertilizer use and reduce runoff. Precision agriculture involves using technology to apply fertilizers more efficiently, based on the specific needs of crops and soil conditions [12]. Additionally, promoting the use of organic fertilizers and soil amendments, such as compost and biochar, can enhance soil health and reduce dependency on synthetic fertilizers [13].

Policy measures are also crucial. Governments can incentivize sustainable farming practices through subsidies and support for research and development in sustainable agriculture technologies. International cooperation is needed to address the transboundary nature of nutrient pollution and to develop global strategies for sustainable fertilizer use [14; 15].

The need for ongoing research is paramount. Current research trends focus on developing enhanced efficiency fertilizers that release nutrients more slowly and in response to plant needs, thereby reducing losses to the environment. Studies are also exploring the potential of nanofertilizers, which use nanoparticles to improve nutrient delivery and uptake by plants [16]. Additionally, research into bioformulations, which combine fertilizers with beneficial microbes, aims to enhance soil health and nutrient availability [17].

Furthermore, establishing long-term fertilization research centres is essential. These centres can provide continuous monitoring and evaluation of fertilizer impacts on soil health, crop productivity, and environmental quality. They can also serve as hubs for developing and testing innovative fertilization strategies and technologies, ensuring that agricultural practices evolve in a sustainable and environmentally friendly manner.

While fertilizers play a vital role in modern agriculture, their ecological impacts are profound and multifaceted. Sustainable management of fertilizer use is essential to mitigate these impacts and ensure the long-term health of both agricultural systems and the broader environment.

The objective of this study was to evaluate the effects of varying nitrogen addition levels on soil ammonium and nitrate pools, as well as on several critical components of nitrogen cycling in soils, including mineralization potential, enzymatic assays of nitrate reductase, and functional microbial groups densities of ammonifiers and denitrifiers.

Materials and methods

Soil samples were taken from topsoil horizon (maximum depth of 20 cm) of agricultural plots managed by the Agricultural Research and Development Station Secuieni (ARDS Secuieni), Neamț County, Romania. This research station has a 49-year experiment with fertilizer additions and crop rotations management (parallel cultures of wheat, corn and bean). Research area soil type is Chernozem soil with a loamy texture. The treatment scheme involves the application of various nitrogen and phosphorus fertilizer inputs, as shown in Table 1, within plots with areas of approximately 30 m^2. Nitrogen additions (inorganic forms as ammonium nitrate) were carried out in spring, while phosphorus was added in autumn. The research of the Secuieni station began in the '70s and is a particularly important reference point in the analysis of the long-term impact of fertilizer application within agricultural systems. Sampling was done in October 2020; each sample was composited from 6 subsamples (three within and three between crop rows) and analysed in triplicate.

Table 1 Fertilizer addition scheme managed by ARDS Secuieni

Sample code	N input (kg/ha/year)	P input (kg/ha/year)
V1	0	0
V3	80	0
V5	160	0
V11	0	80
V13	80	80
V15	160	80
V21	0	160
V23	80	160
V25	160	160

Monitored parameters were determined using freshly collected samples to better understand soil nitrogen biogeochemistry. Moisture content was analysed gravimetrically [18] and pH was determined in the lab using a suspension with distilled water with a 1:4 ratio [19]. Soil organic matter content was also done gravimetrically by placing dried samples in crucibles in an oven set with a combustion temperature of 550°C [20]. Available inorganic nitrogen species (ammonium nitrogen and nitrate nitrogen) were extracted using KCl 0.2 M [21] and after filtration the solutions were used in spectrophotometric methods to assess the nitrogen contents, and results were calculated as micrograms/gram dry weight (µg/g.dw). Orthophosphate levels were also analysed colorimetrically using a soil extract with sodium bicarbonate.

Soil potential mineralization rates were assessed using a two-week incubation in anaerobic conditions at 37°C and extraction with KCl 2M was performed at the end [22]. After filtration, the ammonium nitrogen was spectrophotometrically determined. The initial values for this nitrogen species were subtracted and the mineralization potential was expressed as µg $N\text{-}NH_4^+$/g.dw/day).

We assessed the density of two major functional groups: ammonifiers – responsible with decomposition stage of organic matter and denitrifiers which are involved in soil nitrogen regulation since they ultimately release molecular nitrogen (N_2) back into atmosphere [23]. To estimate the density of selected microbial groups, fresh soil samples were first suspended in sterile physiological saline, inoculated in wells containing specific growth medium (peptone water for ammonifiers and Pochon medium for denitrifiers) and incubated for 24 hours [24]. The inoculation used a three replicates scheme per serial dilution – which used a factor of 10. For denitrifiers the starting dilution was 10^{-1}, while for ammonifiers it was 10^{-2} since they are more abundant in the studied soils. After incubation specific reagents were added (Nessler for ammonifiers and Griess I and II for nitrite detection) and the results were numerically converted using McCrady's table [25] and further reported as individuals/g.dw.

Soil enzymatic assays (nitrate reductase) were performed as presented in Abdelmagid and Tabatabai [26]. Samples were added specific substrate (KNO_3). The method required the use of reference samples (samples stored at -20°C) while assay samples were incubated at 25°C and analysed after 24 hours, using nitrite as byproduct of nitrate reductase activity. For this parameter, the low value range required the nanograms scale, with results being reported as ng $N\text{-}NO_2^-$/g.dw/h.

Results and discussions

Soil water content showed a narrow variation interval, with values ranging from 16.26%-17.00% which can be attributed to similar soil structure and texture and therefore a homogenous water capacity retention of the study area. Water content is normal for October (a cooler season in which frequent precipitation can occur in a temperate climate). A similar homogeneity was found for pH values, which ranged between 5.22-5.57, this distribution implying that soils are moderately to strongly acidic since neutral category is between 6.5-7.5 [27].

Soil organic matter, represented by intermediate products of decomposition processes of biological material, depends mainly on the available substrate for this cycling stage. As far as agricultural systems are concerned, the values are lower compared to a natural system dominated by vegetation, as a significant amount of plant material is removed through crop harvesting. Relatively low and extremely similar values are noted for all selected study areas, with values generally of about 5.5%.

A significant variation observed in the analysed parameters was primarily attributed to nitrogen reserves, particularly ammonium nitrogen (Figure 1). This nitrogen species is less soluble compared to nitrate and therefore less susceptible to percolation and it has a buildup tendency in soils [28]. As expected, the accumulation level is strongly correlated with nitrogen input as fertilizer (R^2 value for the correlation between ammonium nitrogen levels and yearly nitrogen input

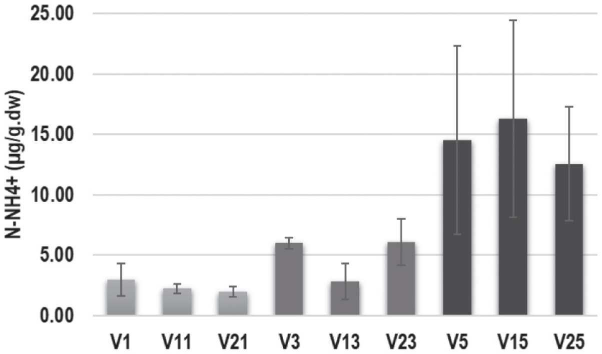

Fig. 1. Soil ammonium nitrogen distribution from Secuieni agricultural complex (October 2020)

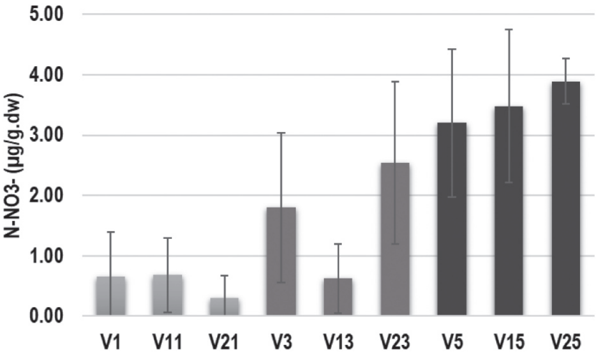

Fig. 2. Soil nitrate nitrogen distribution from Secuieni agricultural complex (October 2020)

of 0 kg, 80 kg and 160 kg is 0.67 – exponential function). For natural systems, ammonium nitrogen is dependent on organic matter content [29], whereas in our study the correlation between ammonium nitrogen and soil organic matter showed a steep decline from 41% in the reference plots to 29% in the intermediate nitrogen addition level (80 kg N/year) to 0.02% in the highest fertiliser input, which further illustrates that ammonium levels are increasingly dependent on nitrogen additions rather than natural cycling drivers.

Similar accumulation trends were observed for phosphorus additions but in this case, no significant difference was reported between the input level, orthophosphate levels reaching a similar plateau for both 80 kg and 160 kg of P per year. This plateau could signal a storage capacity issue, which could pose a risk by transferring excess phosphorus to other ecosystems. This is of special interest since there was little time between fertilization and sampling since phosphorus addition was completed in autumn.

Nitrate levels are significantly lower than the reduced nitrogen species, a situation that can be explained by higher water solubility and losses through percolation, which is one of the key issues in fertilizer management and cultural eutrophication risk [30; 31; 32]. Another contributing factor to lower nitrogen levels in this form is plant assimilation preference for this nitrogen species. This partiality is due *to higher soil mobility* that provides easier root access for plants [33], *energy efficiency* – even if nitrate requires more energy in the absorption stage, it is ultimately easier to metabolize afterwards [34] *synergistic uptake* – nitrate assimilation promotes the concurrent absorption of other necessary elements such as potassium, calcium and magnesium [34] and the *non-volatile* nature of this nitrogen species which makes nitrate a more reliable source of nitrogen compared to ammonium [35]. Accumulation levels across fertilized plots show a strong linear correlation with nitrogen addition (R^2 value is 0.58).

Microbial groups play a crucial role in soil functions and nitrogen cycling, contributing to the overall ecosystems' health and fertility. A key aspect regarding soil microbiota is the capacity to metabolize organic matter, which can be analysed by estimating soil mineralization potential. Since this function is primarily dependent on substrate (available organic matter), there is little data variation for average values due to similar and low values of soil organic matter (Figure 3). However, point by point analysis suggests that for the highest nitrogen addition there is greater data variability, which could infer that decomposing microbial group is responding to excess nitrogen. More stable values are for the control plots as well as for the intermediate addition level of 80 kg N/year, which seems to be a more tolerated addition level. Nevertheless, mineralization rates are slightly lower (by 5.88%) in fertilized plots compared to reference areas.

A more in-depth analysis of soil microbial compartments was performed by using the results of microbial functional group densities. Ammonifier's densities are below 500 individuals/g dry weight except for plots V11 and V25 (Figure 4). Interestingly, there seem to be more ammonifiers in the fertilized plots which could be due to microbial stimulation induced by added nutrients, signalling a reduced competition. Some studies showed that inorganic fertilizers can sometimes diminish the presence of other microorganisms that compete with this functional group for resources, allowing ammonifiers to proliferate [36; 37; 38].

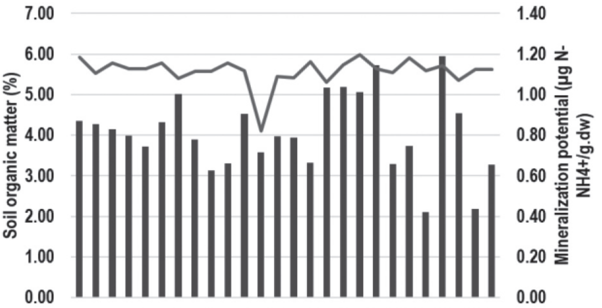

Fig. 3. Distribution of soil organic matter (%) and mineralization potential rates (µg N-NH4+/g.dw/day) from Secuieni agricultural complex (October 2020)

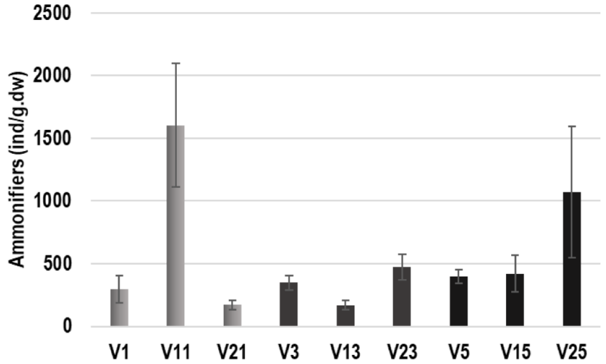

Fig. 4. Soil ammonifiers (ind./g.dw) distribution from Secuieni agricultural complex (October 2020)

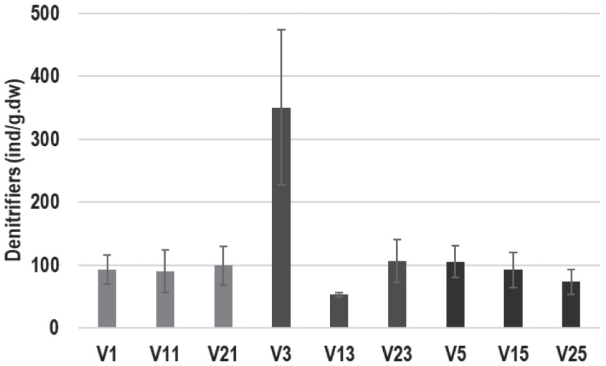

Fig. 6. Soil denitrifiers (ind./g.dw) distribution from Secuieni agricultural complex (October 2020)

Our data shows strong correlations between ammonifying communities and soil ammonium nitrogen for both fertilized plots, whereas there is no correlation for the reference samples between these parameters (Figure 5). Another supporting fact was the correlation between ammonifiers densities and mineralization potential. In this case the trend was descending both for reference samples and highest addition levels, but a very good positive correlation was observed for the samples fertilized with 80 kg N/ha/year, which further suggests that this level of addition is more suitable for soil microbiota health.

Denitrifiers are less abundant than ammonifiers, with ranges five times lower (Figure 6). Only one sampling point (V3) presented an increase in this functional group, but the data regarding nitrate reductase activity does not reflect higher values for this sample. Recent studies proved that inorganic fertilizers could disrupt soil denitrifiers community by reducing both abundance and diversity [39]. A first observation can be made for the fertilized plots that previously shown an accumulation of nitrate, here the densities are generally lower than the reference areas since the primary substrate (nitrate) is more abundant which might indicate a substrate inhibition. Since nitrate is a good substrate for denitrifiers, we also obtained very good correlations between these two parameters. The equations describing this interdependence are logarithmic in nature, suggesting that there is a limiting range of denitrifiers' densities while nitrate levels increase. The degrees of correlation (R^2 values) were: 0.34 for control samples, 0.41 for plots fertilized with 80 kg N/ha/year and 0.32 for those fertilized with 160 kg N/ha/year).

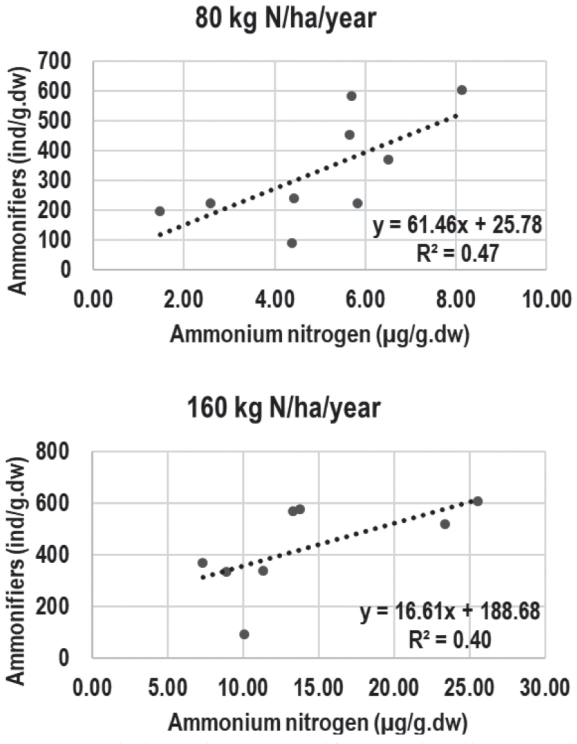

Fig. 5. Correlations for ammonifiers and soil ammonium nitrogen for fertilized plots (top 80 kg N/ha/year; bottom 160 kg N/ha/year from Secuieni agricultural complex (October 2020)

Nitrate reductase activities across samples show a greater value stability for reference samples (Figure 7) and also slight reduction for fertilized plots: control samples have an average value of 29 ng N-NO$_2^-$/g.dw/h, whereas samples from soils fertilized with 80 kg N/ha/year have an average of 25.2 ng N-NO$_2^-$/g.dw/h and those with highest N addition have a mean value of 21.1 ng N-NO$_2^-$/g.dw/h. Though several studies have established that soil enzymatic activity intensifies as a result of fertilization [40], our data suggest a slight decline for this parameter, most likely due to soil pH. It is well established that optimal pH for nitrate reductase activity is between 7.0-8.0 [41]. For instance, one study found that nitrate reduction rates were higher at pH 7.1 compared to pH 5.5, indicating that more neutral or alkaline conditions favour nitrate reductase activity [42]. Another explanation for smaller values of nitrate reductase activity in fertilized plots is the presence of high levels of ammonium, which inhibits nitrate assimilation [43; 44].

Increased nitrate reductase activity can shift the nitrogen cycle towards nitrate reduction rather than denitrification. This shift can reduce the substrate availability for denitrifiers, thereby decreasing their activity and abundance [45].

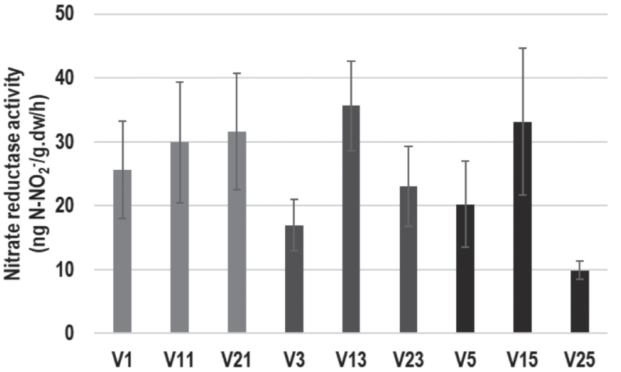

Fig. 7. Average values for soil nitrate reductase activity from Secuieni agricultural complex (October 2020)

Correlations for each replicate samples show a good correspondence between denitrifiers' densities and soil nitrate reductase activity (Figure 8). However, due to lower values from the samples with the highest nitrogen addition, the trend is opposite. These distributions might again suggest that soil microbiota is most disrupted at highest added fertilizer and is more tolerant to the lower nitrogen input.

Conclusions

Inorganic fertilizers, as those used in our research area seem to have a trend of accumulation in soils which is also correlated with input levels. This is especially the case for ammonium with average values for the plots with 80 kg N/ha/year being 4.97 µg $N\text{-}NH_4^+$/g.dw and 14.47 µg $N\text{-}NH_4^+$/g.dw for the 160 kg N/ha/year addition. Nitrate is more soluble and preferred in biological uptake, so for this nitrogen species soil accumulation levels were lower, averaging 1.66 and 3.52 µg $N\text{-}NO_3^-$/g.dw respectively. For control plots there was a good correlation between ammonium and soil organic matter, but the linkage decreased considerably for fertilized soils since the main source in this case was due to fertilizer management and not to natural processes of decomposition.

Nitrogen fertilization induced several changes both to microbial functional groups and to soil processes. Our data shows that soil mineralization potential presented higher data variability, especially for soils under highest nitrogen addition. Ammonifiers' densities seem to be stimulated by added nitrogen, most likely due to suppression of other microbial communities induced by excess nitrogen. Conversely, denitrifying bacteria populations seem to undergo the opposite, a possible explanation being substrate inhibition.

Another parameter that showed variations due to fertilizer addition was soil nitrate reductase activity. Similar to mineralization potential, there is higher data variability, signalling a response to added nitrogen. Average values for nitrate reductase are lower for fertilized soils, the trend being correlated with addition level.

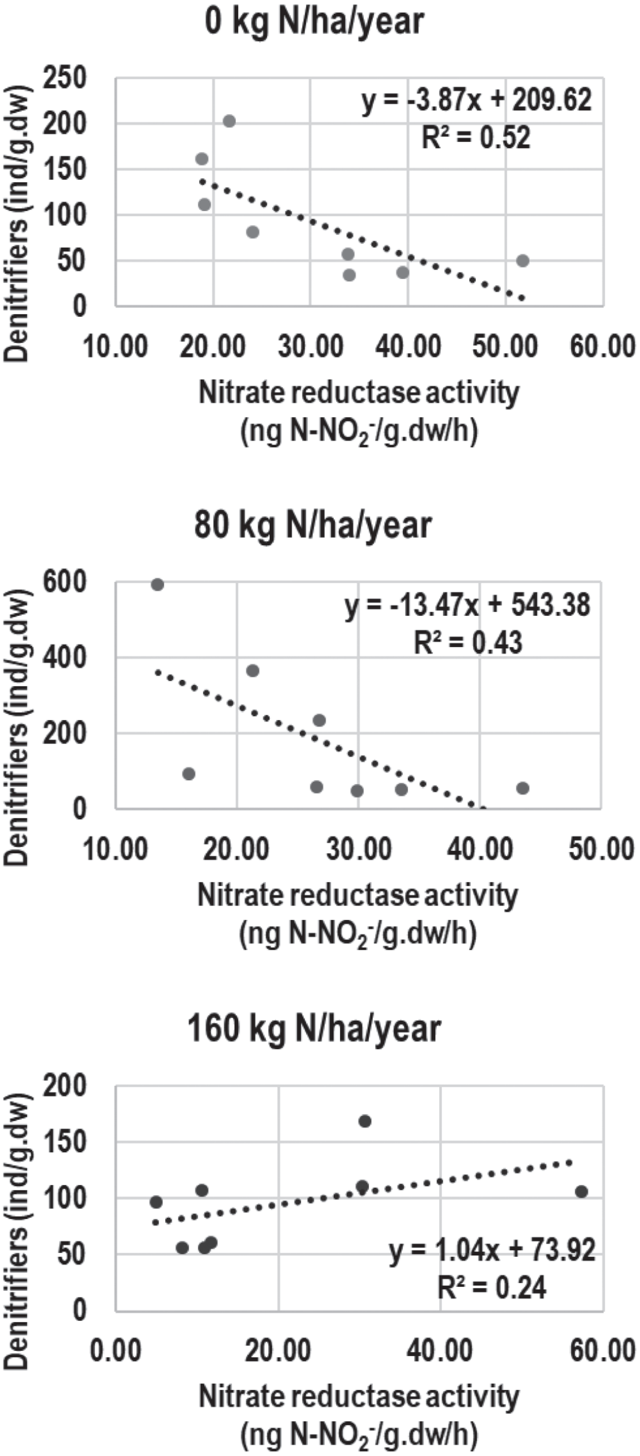

Fig. 8. Correlations for denitrifiers and soil nitrate reductase activities (top 0 kg N/ha/year; middle 80 kg N/ha/year; bottom 160 kg N/ha/year from Secuieni agricultural complex (October 2020)

Our data also suggests that investigated parameters and functions seem to be more resilient to lower levels of N addition (of 80 kg N/ha/year) as compared to values pertaining to nitrogen input of 160 kg N/ha/year.

A very interesting aspect was observed for soil phosphorus levels. Even if the highest addition was double and quite recent to sampling campaign, we found similar soil concentrations for fertilized plots, which suggests that a limit was reached regarding soil storage capacity, and this can pose a significant threat by exporting excess phosphorus to other ecosystems.

Long term research studies are crucial in assessing soil health, quality and productivity of agricultural lands. There are still inefficiencies regarding the balance between fertilizer addition amounts and types (inorganic versus organic) and long-term ecological sustainability. More studies are needed to better understand soils' molecular compartments, their responses, and their variable reactions to fertilizer management practices.

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Conflicts of interest

The authors declare no conflict of interest.

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