



Time series of the nitrogen dynamics after fertiliser application in the topsoil

GEO 511 Master's Thesis

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Abstract

Soil organic matter (SOM) serves as a crucial reservoir for nitrogen (N), with particulate organic matter (POM) and mineral-associated organic matter (MAOM) representing distinct pools characterised by varying turnover times and stabilisation mechanisms. POM acts as a rapid cycling pool, whereas MAOM ensures long-term stabilisation through interactions with minerals that protect organic N from degradation. In this thesis, a density and size fractionation of topsoil SOM was performed to examine ammonium-derived N from fertilisers over a five-year period, providing insights into long-term N stabilisation across SOM fractions. The contrasting behaviour in SOM formation of NH_4^+ -N from cattle slurry (SLU), anaerobically digested slurry (SLA), and mineral fertiliser (MIN) are also analysed. The fertilisers were added in 2018, and the NH_4 -N fraction was ^{15}N labelled. The thesis highlights the reliability of a combined SOM density and size fractionation. Results reveal that MAOM fractions, particularly the fSilt-c fraction, dominate N storage, whereas POM fractions contribute minimally. In the first year after application, the majority of $^{15}\text{NH}_4^+$ -N was poorly stabilised and therefore, the abundance in the fractions decreased rapidly between 2018 and 2019. This leads to the assumption that the ammonium was adsorbed onto minerals and was easily desorbed in the first year (from 2018 to 2019). Recovery and stabilisation of applied N vary with treatments, with SLU showing the highest residual N effect in the soil fractions, emphasising its suitability for long-term retention compared to SLA and MIN. Across all treatments, poorly stabilised N was released rapidly in the first year (from 2018 to 2019), but after 2019 the $^{15}\text{NH}_4^+$ -N abundance stabilised.

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List of Abbreviations

APE	Atomic percent excess	Ndff	Nitrogen derived from fertiliser
BS	Bulk soil	Ndff _{kg/ha}	Nitroge derived from fertiliser in kg per ha
C	Carbon	Ntot	Total nitrogen
cSilt	Coarse silt	NUE	Nitrogen use efficiency
fPOM	free particulate organic matter	oPOM	Occluded particulate organic matter
fSilt-c	fine silt/clay	SLA	Anaerobically digested slurry
MAOM	Mineral-associated organic matter	SLU	Cattle slurry
MIN	Mineral fertiliser	SOM	Soil organic matter
MP	Microplot	SPT	Sodium Polywolframate
N	Nitrogen		

1. Introduction

Nitrogen (N) plays a crucial role in agricultural productivity, as it is often a limiting nutrient in intensive plant cultivation systems (Sradnick & Feller, 2020). N enters the soil through the application of synthetic mineral fertilisers, the recycling of crop residues and farmyard manure, or through the biological fixation of atmospheric N (N_2) by legumes (Sinaj et al., 2017). Sustainable agriculture aims to maximise fertilisers' N use efficiency (NUE) while minimizing environmental and economic losses.

Plants primarily absorb mineral N in forms of nitrate (NO_3^-) and ammonium (NH_4^+) (Figure 1) (Amelung et al., 2018; Sinaj et al., 2017). The processes of fixation, mineralisation, and nitrification increase the available N in the soil, while the processes of denitrification, volatilisation, immobilisation, leaching and N uptake by plants result in a reduction of N in the root zone Sinaj et al. (2017). Mineralisation is the microbial breakdown of organic N (from manure, crop residues, soil N stock, etc.) into ammonia (NH_3) (Figure 1). Nitrification is the conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) by microorganisms (Figure 1). Nitrate is the most available form of N for plants, but it is also highly prone to leaching. Leaching occurs when excess water percolates through the soil, transporting nitrate into groundwater or nearby water bodies (Sinaj et al., 2017) (Figure 1).

Another process that leads to a reduction of plant available N is denitrification, where nitrate is converted to gaseous N compounds like N_2 , NO and N_2O , occurring under anaerobic conditions (Figure 1). Excessive N fertilisation can increase denitrification. Nitrate reduction is pH-dependent and may lead to higher N_2O emissions in acidic soils

(Amelung et al., 2018; Sinaj et al., 2017). Further, ammonia volatilisation reduces the available N and occurs when ammonium-based fertilisers are applied to the soil, especially in the first hours (Figure 1). The loss of ammonia reduces the fertiliser's NUE (Sinaj et al., 2017).

A process that makes N unavailable for plants is immobilisation (Figure 1). It's the opposite of mineralisation, where microorganisms absorb N (nitrate or ammonium) from the soil and incorporate it into their biomass as organic N, temporarily making it unavailable to plants (Sinaj et al., 2017). As microorganisms die and their residues decompose, some immobilised N is remineralised and, therefore made available to the plant again. (Sørensen & Amato, 2002).

Adding fertilisers to the agricultural fields is essential to replenish nutrient levels in the soil that are depleted through the continuous removal of plant biomass from the field. The use of N fertilisers, whether organic or inorganic, can improve yields, however their effect on N recovery in crops depends on proportion of plant available N, decomposability of organic N and N

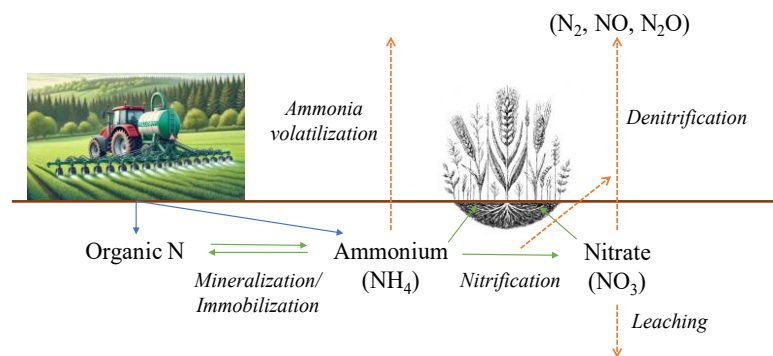


Figure 1: Visualisation of the most important N transformations and losses after application of animal manure to soil. Small images created by KI inspired by the depiction in Webb et al. (2011).

losses during the application (Bosshard et al., 2009; Frick et al., 2023). Mineral fertilisers consist solely of inorganic N forms (NO_3^- and NH_4^+), which are directly available to plants, resulting in a high NUE during the first year of application (Fuchs et al., 2023).

In contrast, N from organic fertilisers (manure, cattle slurry, digested cattle slurry, compost, ...) contains 30-75% organic N (Fuchs et al., 2023), which is not plant-available immediately and primarily enters the soil organic N pool (Gutser et al., 2005). Organic N mainly consists of complex molecules from resistant components in faeces or litter (Webb et al., 2011). This organic N must first partially undergo mineralisation to become plant-available (Fuchs et al., 2023). Not only do organic fertilisers supply essential nutrients to plants, but they also contain readily degradable carbon (C), serving as an energy source for soil microorganisms. These microorganisms play a key role in immobilising N from both the soil and the applied fertiliser (Petersen, 2006). When the carbon-to-nitrogen (C/N) ratio is wide, meaning there's a high C content, a portion of the fertiliser's N becomes immobilised in the soil's N pool (Webb et al., 2011). Consequently, there is a negative correlation between the C/N ratio and net N mineralisation (Sradnick & Feller, 2020).

Due to the slow-release through mineralisation of organically bound N and the medium - to long - term immobilisation of N in soils, the direct N availability of organic fertilisers in the year of application is therefore limited (Gutser et al., 2005). Synchronising N supply from organic manures with crop N demand is challenging. High NUE requires sufficient N during peak plant demand and minimal N during low demand. The soil microbial activity that mineralises organic N is not always well synchronised with plant needs since it is heavily dependent on environmental conditions such as soil moisture and soil temperature (Möller & Müller, 2012). During mineralisation and immobilisation, N losses can occur that cause harm to the environment and result in a loss of productivity (Fuchs et al., 2023). Nevertheless, organic fertilisers are a valuable alternative to mineral fertilisers as they can help to close the nutrient cycles on the farm while increasing long-term soil fertility (Fuchs et al., 2023).

To facilitate better synchronisation between N supply and crop N demand, anaerobic digestion of animal manure can be a possibility (Möller et al., 2008). This process involves the anaerobic (absence of oxygen) breakdown of biodegradable feedstocks such as animal waste (van Midden et al., 2024). During anaerobic digestion, C present in the feedstock is converted into methane, which can be collected as biogas and C dioxide (van Midden et al., 2024). Through the anaerobic digestion of organic matter, organic N is partially mineralised by microorganisms into plant-available ammonium (van Midden et al., 2024). Also, easily decomposable compounds, such as volatile fatty acids, which lead to N immobilisation when organic fertiliser is applied to soil, are broken down during the treatment process (Webb et al., 2011). The produced digestate thus serves as a quick-acting

Table 1: Impact of anaerobic digestion on indicators used to describe the plant N availability from organic fertiliser. (Modified from Gutser et al., 2005).

		Impact of anaerobic digestion
Organic substance	amount	decrease
	stability	increase
C/N ratio		decrease
NH_4^+ - N		sharp increase
pH value		sharp increase

organic N fertiliser where the mineralisation is partially already carried out by the microorganism in the biogas plant before the application (Jarosch et al., 2018; van Midden et al., 2024). Nevertheless, significant amounts of organic N remain in the digestate (Jarosch et al., 2018), resulting in still smaller amounts of inorganic N compared to mineral fertiliser (Möller & Müller, 2012). Due to their immediate fertiliser effect, digestates are easier to synchronise with plant demand than undigested organic fertiliser (van Midden et al., 2024). In Table 1, the changes in different characteristics of organic fertilisers after anaerobic digestion are listed. As described before, the ammonium to total N ratio increases, whereby the C/N ratio decreases because of the decrease of C content. Furthermore, the pH value increases and the viscosities decrease after the digestion (Möller & Müller, 2012). Because of the increase in pH and $\text{NH}_4\text{-N}$, the potential for NH_3 losses during storage and application increases. However, the emissions after application are reduced because of the quicker infiltration into the soil due to the reduced dry matter content (Webb et al., 2011).

The application of organic fertilisers is crucial for maintaining long-term soil fertility and organic matter stability (Sørensen, 2004). The remineralisation of microbially immobilised N, along with the delayed mineralisation of organic N, results in residual N effects that extend beyond the year of application (Schröder et al., 2013). Petersen and Sørensen (2008) estimated that the residual effects of manure-N were equivalent to 2–8% of the total N applied in the year following application. With annual applications, these residual effects accumulate over time (Schröder, 2005). To maximise the benefits of organic fertilisers, it is vital to understand the amount of N mineralised both in the application year and in subsequent years (Berntsen et al., 2007). Consequently, the true fertiliser value of organic fertilisers can only be accurately assessed by considering their long-term effects (Schröder, 2005).

It is also important to understand the dynamics of the N pools in the soil, how and where the remaining N that is not taken up by the plants or lost is stored. Soil organic matter (SOM) serves as a reservoir for N, so it's important to understand how it forms and interacts with fertilisers to optimise N fertilisation (Fuchs et al., 2023).

Particulate organic matter (POM) consists of slightly decomposed plant material that partially consists of N and has a rapid turnover time (Jilling et al., 2018). The free POM (fPOM) is barely decomposed since it consists of mainly plant residues and/or dead roots. Occluded POM (oPOM) is further decomposed plant material and occluded within soil aggregates of different sizes and has therefore a longer turnover time (Griepentrog & Schmidt, 2013; Kim et al., 2022; von Lützow et al., 2007). Aggregates are combinations of minerals and organic compounds that reduce microbial access to the organic matter, making it more stable (Angst et al., 2021).

Organic matter can also be stabilised in the soil by interacting with minerals, forming stable organo-mineral complexes via ligand exchange or Van der Waals forces (Amelung et al., 2018; Angst et al., 2021). Clay particles, and to a lesser extent silt, play a central role in this process due to their abundant reactive oxide and phyllosilicate surface (Angst et al., 2021). This mineral-associated organic matter (MAOM) protects organic N from rapid breakdown and is therefore temporarily unavailable for plants (Amelung et al., 2018). For the mineral-associated stabilisation of organic N, there are two possible pathways. The microorganism incorporates residues and POM, which results in dead microbial matter and other microbial by-products that

bind to minerals. The other pathway is that low-molecular-weight compounds, released when enzymes break down POM or from root exudates, get attached to soil minerals (Manzoni & Cotrufo, 2024).

Ammonium can also directly bind to clay. Clay minerals have negative charges that are balanced by cations, such as NH_4^+ (or Ca^{2+}). Once the NH_4^+ ions penetrate into the clay mineral interlayers, they are excluded from nitrification, thus being protected from leaching (Nieder et al., 2011). Therefore, the soil clay content significantly impacts N retention due to its binding capacity (Fuchs et al., 2023). MAOM, in contrast to POM, is a more physically and chemically stabilised pool and is more resistant to degradation and has therefore a longer mean residence time (Kim et al., 2022). Recent research nevertheless, has highlighted the dynamic nature of these associations, suggesting that MAOM is not merely a passive reservoir of N but plays an active role in regulating N availability. For instance, Jilling et al. (2018) emphasise that MAOM is enriched in low-molecular mass plant compounds and microbial by-products, resulting in a low C/N ratio that generally promotes N mineralisation. In contrast, POM often has a higher C/N ratio and can therefore act as a N sink during early decomposition stages (Jilling et al., 2018).

Fuchs et al. (2023) found that two years after applying ^{15}N labelled mineral or organic fertilisers in the field, most of the N was stored in the MAOM fraction and that most of the N in the plants originated from MAOM, which supported the theory of Jilling et al. (2018). The study further found that N from slurry and mineral fertilisers behaves similarly in soils over time. This master's thesis aims to extend the study of Fuchs et al. (2023) and provide more insights into the long-term impacts of N management strategies, contributing to more sustainable and efficient agricultural practices. Therefore, the dynamics between the SOM fractions are examined to gain a better understanding of the long-term stabilisation of N in the soil.

This master's thesis will address the following research questions:

1. How do the dynamics of NH_4^+ -N derived from fertiliser change over a period of 5 years in the topsoil?

Hypothesis: The amount of fertiliser NH_4^+ -N will decrease rapidly in the first year after application, followed by a decline in the rate of decrease.

2. How does the amount of NH_4^+ -N derived from fertiliser change over time in the different SOM fractions?

Hypothesis: The proportion of N derived from fertiliser NH_4^+ in POM decreases over the study years and the proportion of fertiliser-N in MAOM will slightly increase.

3. What are the differences in NH_4^+ -N dynamics between cattle slurry and anaerobically digested slurry?

Hypothesis: After 5 years, more fertiliser NH_4^+ -N will be detectable in the SOM with the application of undigested slurry compared to digested slurry.

2. Materials & Methods

2.1 Field experiment

The samples that were used for the thesis were collected as part of the Recycle4Bio project, which consists of a field experiment at 330 m a.s.l. in Wallbach, northwestern Switzerland. The aim of the project was to fill the knowledge gaps of the NUE of liquid and solid organic fertilisers under conditions of organic farming in Switzerland in long-term field experiments (Bünemann-König & Mayer, 2021). The field has been under organic management since 2017. During the experiment, normal farm machinery for all operations such as tillage, seeding, weeding and harvesting, have been used (Bünemann-König & Mayer, 2021). The mean annual temperature between 2012 and 2022 was 11.1°C and the mean annual precipitation was 1192.2 mm, as recorded by the weather station nearby in Wallbach (Agrometeo). The mean annual precipitation in the experimental years 2018 and 2019 was very low, with 534.2mm in 2018 and 424.2mm in 2019. There was a long dry period in March and April 2020 (Figure 2).

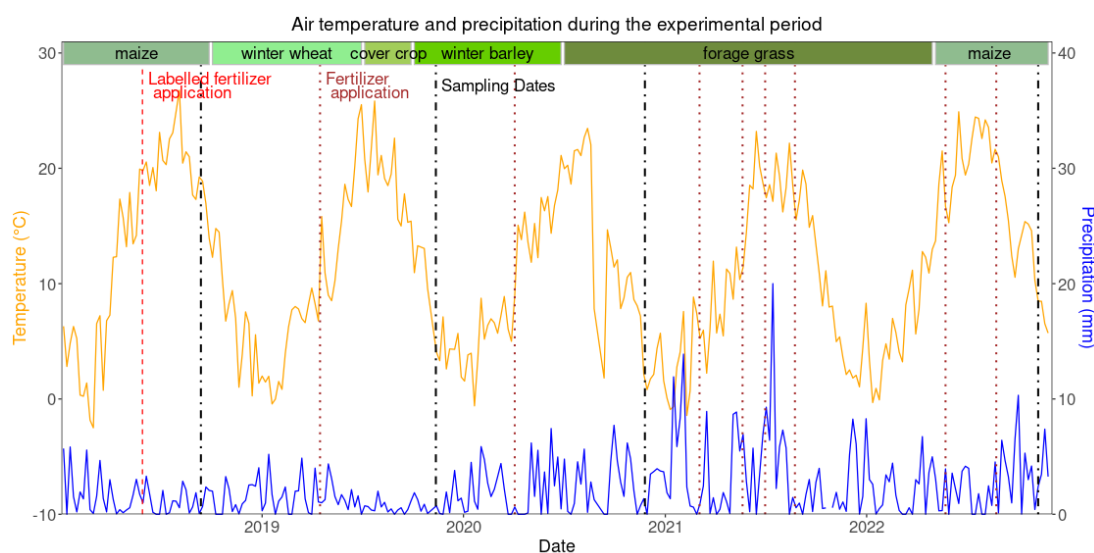


Figure 2: Air temperature (orange, left y-axis) and precipitation (blue, right y-axis) during the experimental period. (Data from weather station near Wallbach (Agrometeo)) The green boxes indicate the crops in the field during these time periods. The red line (dashed) indicates the time point when the labelled fertilisers have been applied and the two brown (dotted) lines when fertiliser without labelled N was applied. The three black lines (dotdashed) indicate the Probing Dates of the used samples.

In 2018, maize was cultivated, followed by winter wheat and mustard as an intercrop in 2019. Winter barley was grown in 2020 and after that, forage grass until 2022. Finally, in spring again, maize was cultivated (see Figure 2). The soil was ploughed to a depth of 20 cm before each main crop. Due to a prolonged drought in the summer of 2018, silage maize required one irrigation in early August (Bünemann-König & Mayer, 2021).

The soil is classified as Haplic Luvisol, with a silt loam texture and a pH of 5.6 (Table 2) (Bünemann-König & Mayer, 2021).

For this thesis, 3 treatments were used, each involving four replicates. All fertiliser treatments received the same total N application: 140 kg N ha⁻¹ for maize and winter wheat and 120 kg N ha⁻¹ for winter barley (Bünemann-König & Mayer, 2021). All fertilisers were spread manually, using watering cans to simulate a drag hose. The samples analysed in this thesis include the four replicates from the mineral fertiliser (MIN), cattle slurry (SLU), and anaerobically digested slurry (SLA) treatments. Only the topsoil (0-20cm) was examined. The samples that are used were taken in autumn in the years 2018, 2019, 2020 and 2022 with a Pürckhauer auger (Bünemann-König & Mayer, 2021).

Ammonium sulfate has been taken as a mineral fertiliser, SLU was sourced from an organic farm (Aemethof, Densbüren) in 2018 and 2019, but in 2020 it was diluted with rainwater and replaced by slurry from a conventional farm (Paul Frey, Asp). SLA was obtained from Syn-farms, an agricultural biogas plant that processes cattle slurry, solid cattle and horse manure, and various plant-based food processing wastes, including residues from coffee capsules, chocolate, and herbal drops. This facility employs thermophilic anaerobic digestion at 53°C with a mean residence time of 60 days, followed by solid-liquid separation, with SLA being the liquid fraction (Bünemann-König & Mayer, 2021).

As described in Table 1 the values of pH and the percentage of NH₄⁺ from total N are higher and the C/N ratio lower for the SLA compared to SLU. The dry matter content and total N values are also higher for SLA (see Table 3).

Table 3: General characteristics of the applied organic fertilisers (mean ± stdev of 6 values) (Bünemann-König & Mayer, 2021).

Fertiliser	Dry matter content (%)	pH	Total N (g/kg)	NH ₄ ⁺ (% of total N)	C/N ratio
SLU	4.6 ± 1.4	7.1 ± 0.2	1.9 ± 0.4	49.6 ± 3.9	10.3 ± 1.3
SLA	5.5 ± 0.8	7.9 ± 0.1	4.2 ± 0.8	62.0 ± 4.7	5.4 ± 0.9

2.2 ¹⁵N labelling

In 2018, microplots (MP) (3 x 2.25m) were labelled with ¹⁵N-enriched NH₄⁺-N within the field (Bünemann-König & Mayer, 2021). In the year of application and the following, the residual value of the ¹⁵N labelled fertiliser was investigated (Bünemann-König & Mayer, 2021). All fertilisers (SLU, SLA, MIN) were labelled immediately before field application by adding a tracer solution containing (¹⁵NH₄)₂SO₄ (99 atom% ¹⁵N) to enrich the NH₄⁺-N fraction of the

Table 2: General soil characteristics (0-20 cm) in February 2018. Means ± standard deviation of all initial plots (Bünemann-König & Mayer, 2021).

Parameter	Mean ± Stdev
Sand	30 ± 7 %
Silt	52 ± 6 %
Clay	18 ± 1 %
pH _{CaCl2}	5.6 ± 1
Total organic C	15.3 ± 0.8 g kg ⁻¹
Total N	1.6 ± 0.1 g kg ⁻¹
Microbial C	403 ± 47 mg kg ⁻¹
Microbial N	70 ± 7 mg kg ⁻¹

fertilisers to 7 atom% ^{15}N excess. The required amount of fertiliser for all four replicates was filled into a barrel, the tracer solution was added, and thoroughly stirred. Each MP18 (MP of the year 2018) was then fertilised individually with the treatment-specific volume of liquid organic fertiliser to apply $140 \text{ kg total N ha}^{-1}$, as in the main plot experiment (Bünemann-König & Mayer, 2021).

2.3 Sample preparation

In the laboratory, a combined SOM density and size fractionation (Fuchs et al., 2023) was carried out. The method is adapted from Steffens et al. (2009) and is a combination of density and particle size fractionation, which distinguishes five SOM fractions (Fuchs et al., 2023).

For the density fractionation, 10g of 2mm sieved, air-dried soil sample was weighed and added to a Sodium Polytungstate solution (SPT) as heavy liquid for separation ($451\text{g SPT}/411\text{g H}_2\text{O}$; 1.8 g/cm^3). First, 30ml of the SPT solution was filled into a tube, followed by the soil sample. The mixture was left overnight without shaking. FPOM that floats above the SPT (visible in Figure 3 in the image of the first step) was then extracted with a pipette and vacuum-filtered to separate it from the SPT solution.

For aggregate dispersion, bi-distilled water (30ml) was added to the mixture, and then the solution was treated with ultrasound (Sonoplus UW 2200 Ultrasonic transducer, Bandelin Electronic, Berlin, Germany) for 7 minutes and 34 seconds, providing a dispersion energy of 100 J/ml (Figure 3 second image). The Sonotrode (Model VS 70T) was immersed 20mm into the solution. During the sonication, the soil was cooled with ice to avoid overheating. Afterwards, the solution was centrifuged at 4600 rpm for 15 minutes, and the water was decanted. The solution was then refilled with SPT and shaken before being centrifuged again at 4600 rpm for 30 minutes. The vacuum filtering steps were repeated as for the fPOM for the occluded particulate organic matter (oPOM). For wet sieving, the remaining sample was first also vacuum filtered to extract the remaining SPT and passed through $63\mu\text{m}$ and $20\mu\text{m}$ sieves with bi-distilled water, until the liquid was clear, resulting in coarse silt (cSilt), fine silt/clay (fSilt-c), and sand, which were categorised as MAOM (Figure 3 third image). The fractions were then dried at 60°C in an oven, and before weighing, they were dried again overnight. Each

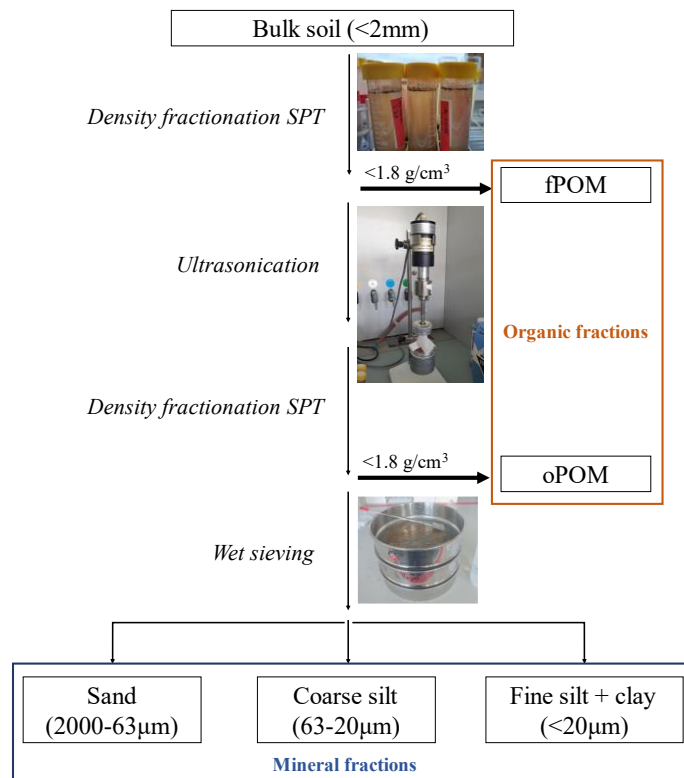


Figure 3: Density-size fractionation scheme adapted from Fuchs et al., (2023). Images of the fractionation steps are self-made.

fraction was weighted for its total mass. Finally, the dried samples were weighed in tin capsules, with the following amounts prepared for ^{15}N analysis: bulk soil 40mg, Sand 100mg, fSilt 80mg, cSilt 80mg, and fPOM/oPOM 5mg.

The analysis of total N and ^{15}N in the soil samples of the fractions and the bulk soil (BS) was conducted using an elemental analyser (PYRO cube, Elementar, Germany) coupled with an isotope ratio mass spectrometer (isoprime precision, Elementar, Germany). All the lab work was performed by myself, whereas the analysis on the IRMS was performed by Martin Zuber from the Environmental Analytics group at Agroscope Reckenholz.

2.4 Calculations

The soil samples of the different fractions and from BS were analysed for total C, total N and ^{15}N abundance. To calculate the mass of total N (N_{tot}) in each fraction the measured N in percent was multiplied with the mass that resulted from each fraction.

The percentage of N derived from the $^{15}\text{NH}_4^+$ -labelled fertiliser (Ndf%) served as an indicator of how much of the N_{tot} in each soil sample originated from the applied fertiliser. It was calculated following (Hood et al., 2000):

$$Ndf\% = \frac{^{15}\text{N}_{\text{APE}}}{^{15}\text{N}_{\text{APE}_{\text{fertiliser}}}} * 100$$

where $^{15}\text{N}_{\text{APE}}$ represents the atom% ^{15}N excess of the soil sample (^{15}N abundance of the sample minus the natural abundance), and $^{15}\text{N}_{\text{APE}_{\text{fertiliser}}}$ the atom% ^{15}N excess of the labelled fertiliser (7% plus natural abundance).

Furthermore the NH_4^+ derived from fertiliser in mg in each fraction was calculated following (Hood et al., 2000):

$$Ndf_{\text{mg}} = \frac{Ndf\%}{100} * N_{\text{tot}}[\text{mg}]$$

This amount of NH_4^+ -Ndf in the fractions was extrapolated to kg per ha ($Ndf_{\text{kg/ha}}$):

$$Ndf_{\text{kg/ha}} = \frac{Ndf_{\text{mg}}}{10\text{g}} * 1\text{ha} * \text{Depth (m)} * \text{Density} \frac{\text{g}}{\text{cm}^3}$$

where the 10g indicates the mass of the soil sample before fractionation, the volume of a ha of soil with 0.2m depth and the density of the soil for each plot.

The recovery (%) of the NH_4^+ -N in the different fractions was calculated as follows (Hood et al., 2000):

$$Ndf_{\text{recov}\%} = \frac{Ndf_{\text{kg/ha}}}{\text{NH}_4^+ - N_{\text{applied}(\frac{\text{kg}}{\text{ha}})}} * 100$$

with NH_4^+ - N_{applied} representing the amount of NH_4^+ -N initially applied to the plot in kg/ha.

2.5 Statistical analyses

Calculations and statistical analyses were conducted using Rstudio (R Core Team, 2025) coupled with R version 4.4.3. Throughout all statistical analyses, a significance of $p < 0.05$ was used. To compare the differences between the years, a Friedman-test was used, unless the residuals were normally distributed (then with repeated measures ANOVA). Differences between the treatments or fractions were analysed using a Kruskal-Wallis test, unless the residuals were normally distributed, then ANOVA was applied.

After a visual identification of some outliers in the boxplot, the Z-score method was applied to identify outliers in the data. The Z-score method is based on calculating the Z-value for each data point. The Z-value indicates how many standard deviations a value is from the mean of the data. Data points with an absolute Z-value greater than 3 are considered outliers. Through this analysis, 4 outliers in the $\delta^{15}\text{N}$ data were detected: one in the fPOM fraction in 2018, one of the sand fraction in 2019 and two from 2022 one oPOM fraction and the other from the fPOM fraction. These 4 results were excluded from further analysis.

3. Results

3.1 Assessment of method performance

The recovery of the measured parameters in the fractions compared to these parameters in the BS provides an understanding of how well the fractionation method performed.

The combined mass of the different fractions ranged from 94.6% and 98.5% of the 10g of the BS used for the fractionation (Figure 4). The fraction with the smallest mean mass was the oPOM, making up only 0.18%, followed by fPOM with 0.19%. These two fractions did not differ significantly between 2018 and 2022. The majority of the mass was from the mineral fraction, with the largest share belonging to fSilt/clay, which had an average proportion of 42%. In 2022, the mass proportion of cSilt (mean: 39%) exceeded the mass proportion of fSilt-c (mean: 37%) in all the treatments. The mass of the mineral fractions did change significantly between 2018 and 2022 (sand: $p = 0.003$, cSilt: $p = 0.036$; fSilt-c: $p < 0.001$). The mass proportion of the fSilt-c decreased (-6.08%), whereas the sand and cSilt mass proportion increased (+1.6%/ + 2.96%) over the 5 years.

After fractionation, between 92.6% and 102.1% of the N_{tot} in the BS was recovered in the fractions (Figure 5). Thereof, $87.5 \pm 3\%$ is recovered in the fSilt-c fraction and $93.4 \pm 3\%$ in MAOM in general. The recovery of the ^{15}N abundance of BS was in the fractions across the treatments between 68% and 95%. Figure 6 indicates that the recovery rate in 2018 was lower than in the following years. In that year, MIN experienced the most significant loss, averaging a 31.86% reduction during the fractionation process.

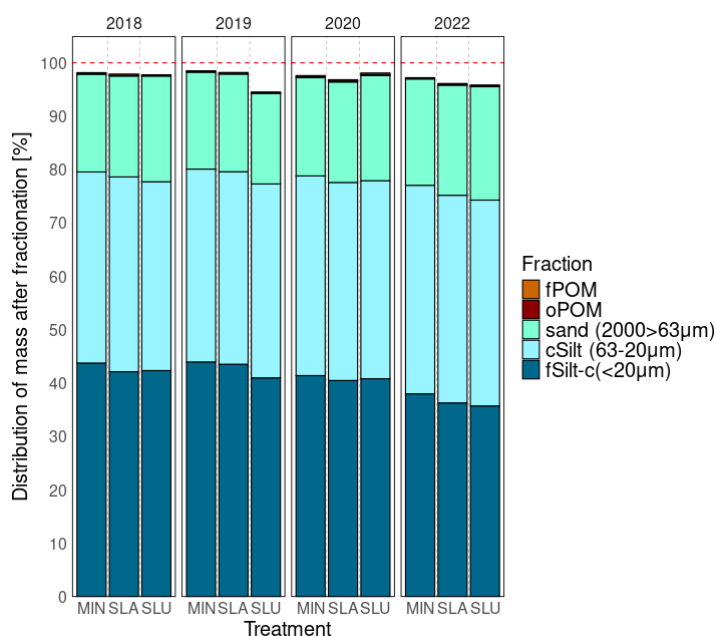


Figure 4: Recovery of the soil mass in the different fractions as a percentage of the mass of the BS sample before the fractionation.

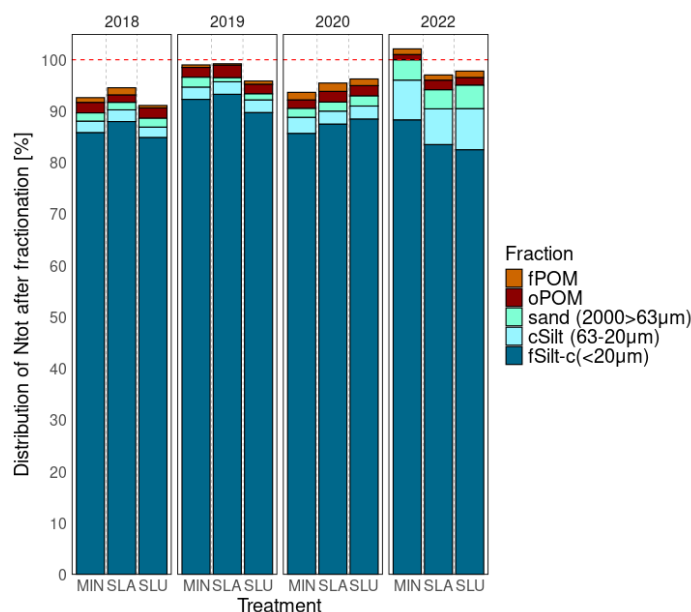


Figure 5: Recovery of the total nitrogen in the different fractions as percentage of the nitrogen derived from the fertiliser in the BS before the fractionation.

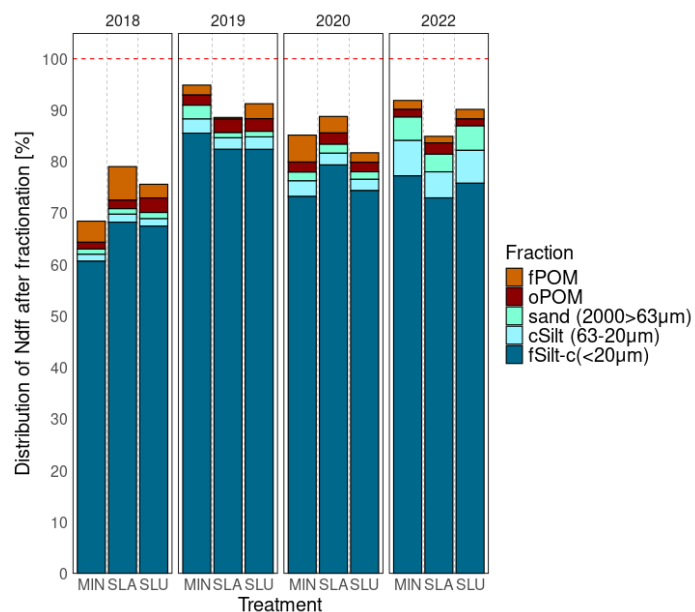


Figure 6: Recovery of the nitrogen derived from the fertiliser in the different fractions as percentage of the nitrogen derived from the fertiliser in the BS before the fractionation.

3.2 Characteristics of fractions

The distribution of Ntot across soil fractions shows notable stability, with little variation observed over the years between fractions and within fractions. The C/N ratio helps to understand the dynamics of N in the soil and assess the long-term effects on N availability.

The highest N concentration of Ntot shows the oPOM fraction, followed by the fPOM fraction (Figure 7). There is a high data scattering in the POM fractions because these fractions are less homogeneous than the MAOM fraction. Although the POM fractions have a high N concentration, their contribution in absolute N is very small because their mass is very small in comparison to the fSilt-c fraction for example, where the most Ntot is stored (Figure 5). The values of Ntot% in the BS are very stable in all years and all treatments and do not change significantly. Ntot% in the fractions are also very stable between treatments (Figure 7), but there are significant differences in the MAOM fractions between the years (sand: $p = 0.045$, cSilt: $p = 0.032$, fSilt-c: $p = 0.01$). The data shows a small change of Ntot in the sand and coarse silt fraction in the year 2022, in which the N concentration is for all treatments higher than in the other years (Figure 7). The Ntot% of fSilt-c also increases over the years.

The C/N ratio of the BS and the fSilt-c fraction is very stable over all years and between all treatments. For BS, the mean C/N ratio is 10.39, with a standard deviation of 0.51 ($n=48$). The mean C/N ratio for fSilt-c is 8.68 ± 0.31 . Especially in the fPOM fraction, there is a high variance in the C/N ratio (mean: 31.75 ± 13.36), but it does not differ significantly between treatments ($p = 0.434$). The oPOM fraction has the second highest C/N ratio with a mean value of 24.41 ± 4.33 . The change in 2022 of the Ntot% value in the cSilt and sand fraction is also visible in the total C concentration data. Nevertheless, the Ntot% value in cSilt increases more than the

total C concentration because the C/N ratio is lower in 2022 compared to previous years. The mean values of these two fractions are very similar (sand: 19.82 ± 7.02 , cSilt: 19.84 ± 4.92).

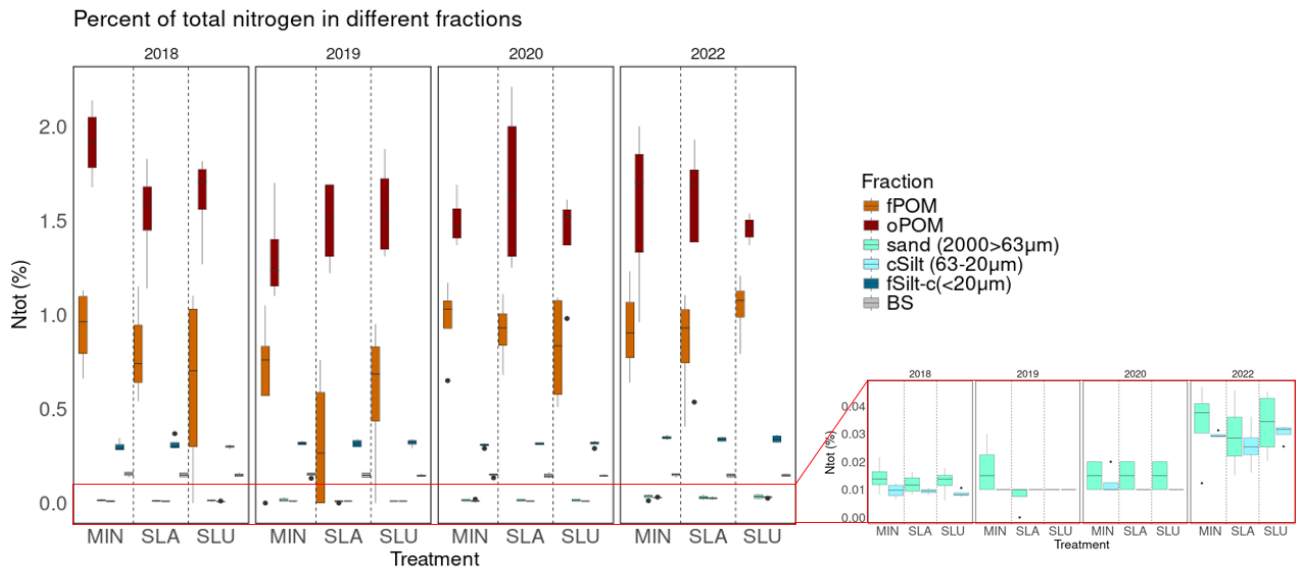


Figure 7: Total nitrogen in different fractions as well as BS in percent. The big boxplot shows all the fractions and the small one on the right is a zoom in of the sand and coarse silt fraction.

3.3 Nitrogen derived from fertiliser

The ^{15}N atom percent excess and Ndff provide valuable insights into how applied N is distributed and stabilised in soil over time. By analysing these parameters, it becomes possible to assess changes in N cycling, including the impact of different treatments on N retention across the soil fractions.

The proportion of atom% ^{15}N excess and therefore, also the Ndff decreases in all fractions over the period of 5 years for all treatments. The change of Ndff% between 2018 and 2022 in the oPOM fraction ($p = 0.058$) and the sand fraction ($p = 0.308$) is not significant. The highest percentage of Ndff% over all years and treatments was found in fPOM, whereas all the other fractions show a similarly lower value. The Ndff% in fPOM differed between treatments, the highest value was for MIN followed by SLU and then SLA in all the measurement years. Although this difference is visible in Figure 8 (the order of magnitude of Ndff% is the same as atom% ^{15}N excess of the different fractions), the values between the treatments do not differ significantly ($p = 0.235$). Just in the year 2020, the Ndff% differed significantly between the treatments in the MAOM fractions ($p < 0.05$). Same as in the Ntot values, fPOM has, despite its high Ndff% value, a very low influence in the absolute value Ndff_{kg/ha} (Figure 9) because of the low mass proportion. As a mean of all 4 years, 72% of the Ndff is stored in the fSilt-c fraction.

Looking at the absolute value of Ndff_{kg/ha} in the BS, a rapid decrease between 2018 and 2019 is visible in Figure 9. After 2019, the values stabilised and decreased just a bit. The changes in BS show a strong correlation with the change in fSilt-c (Pearson-coefficient: 0.99 for all treatments). Also, Ndff_{kg/ha} in the fPOM fraction shows a strong decrease in the first year. The

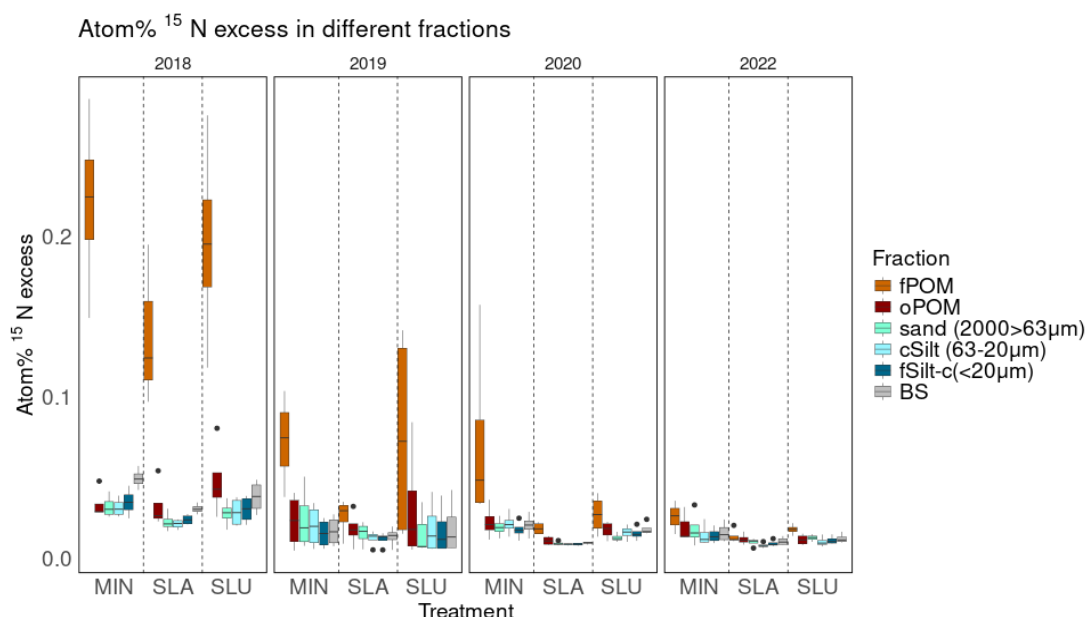


Figure 8: Atom% ^{15}N excess in the different fractions.

change in $\text{Ndff}_{\text{kg/ha}}$ between 2018 and 2019 is just for the BS, fSilt-c and fPOM fraction significant for the data of all treatments. Looking at the $\text{Ndff}_{\text{kg/ha}}$ for each treatment separately, the MIN and SLA change significantly as said between 2018 and 2019. However, the decrease in $\text{Ndff}_{\text{kg/ha}}$ for SLU is not significant during this period, it only becomes significant when comparing the values from 2018 to 2022.

Between 2019 and 2022, just the sand and cSilt fraction changed significantly ($p = 0.0212$; $p = 0.0369$). The data shows that these two fractions have a small decrease between 2018 and 2019 but increase a little bit in the following years. These two fractions had the highest values in 2022. This results in total in a small increase over the 5-year period in the sand and cSilt fractions for all treatments. The highest increase was in the cSilt fraction and MIN, with an increase of 59% of the $\text{Ndff}_{\text{kg/ha}}$ in 2018.

The highest decrease in $\text{Ndff}_{\text{kg/ha}}$ over the study period was for MIN compared to SLU and SLA, with a decrease from all fractions together of -11.55 kg/ha. 91.98% of this decrease is from the fSilt-c fraction. For SLU, the fSilt-c fraction contributed 92.34% to the change between 2018 and 2022. The lowest change was for SLA with -9.17 kg/ha. However, the differences between the treatments are not significant (for all fractions $p > 0.05$).

Between 2018 and 2019, already 93.73% of the decrease between 2018 and 2022 of $\text{Ndff}_{\text{kg/ha}}$ occurred for MIN. SLU has, in comparison to the other fertilisers, the smallest change in the period 2018 to 2019, with a decrease of 68.55% of the total decrease over the 5 years.

The $\text{Ndff}_{\text{kg/ha}}$ of oPOM for SLU in 2018 was higher than for the other treatments. This difference is nevertheless not significant. A significant difference between the treatments is found in the cSilt and fSilt-c fractions in the year 2020 ($p < 0.05$). MIN and SLU have a small increase in the median in this year, whereas in SLU, the $\text{Ndff}_{\text{kg/ha}}$ value decreases further, leading to this significant variability between the treatments. Not a significant difference between the treatments is the increase in $\text{Ndff}_{\text{kg/ha}}$ of fPOM and BS from MIN in the same year (visible in Figure 9).

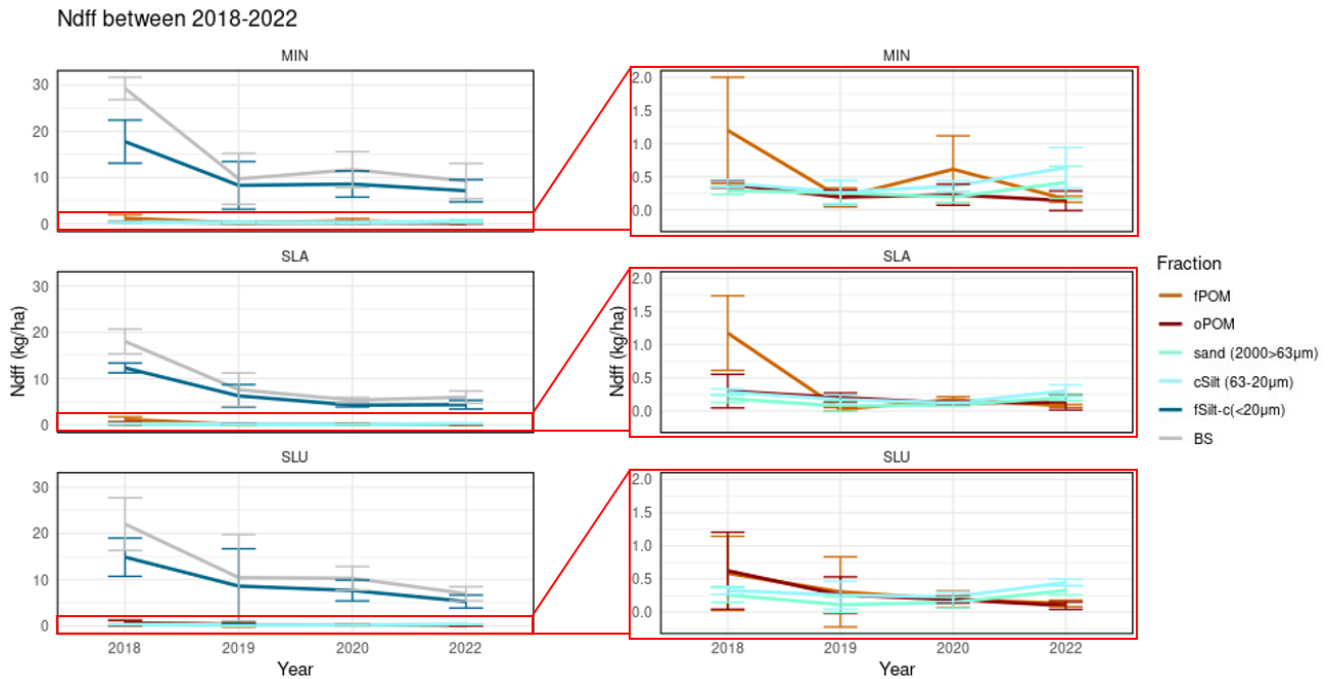


Figure 9: Course of the absolute values of N derived from fertiliser over the 5 years period. In the left figure all fractions plus the BS are shown whereas in the right figure the fractions with values between zero and two kg N per ha are zoomed out. The error bars represent the standard deviation ($n=4$).

3.4 Recovery of added fertiliser

To understand the amount of NH_4^+ derived from the different fertiliser types, Ndff was normalised by the amount of $\text{NH}_4\text{-N}$ applied with each fertiliser treatment (Table 3). This gives us the percentage of how much of the applied ammonium can still be found in the soil fractions.

In all treatments, the recovery of the applied $\text{NH}_4^+\text{-N}$ has approximately halved between 2018 and 2019 in the sum of all fractions and is quite stable, especially for MIN and SLA after 2019 (Figure 10). The highest percentage of the applied $\text{NH}_4^+\text{-N}$ was found in the fractions for SLU in 2018, with a mean N recovery in sum of all fractions of $24.37 \pm 6.4\%$. SLA has just a slightly higher mean recovery in the fractions in 2018 than MIN. In 2020, the recovery in MIN was higher than in 2019 and was also higher than the recovery of SLA. Similar to MIN, the SLU values in 2020 show an increase in median recovery across all fractions; however, this increase is not reflected in the mean of the four replicates. More clearly, the data shows an increase of the N-recovery in the sand and cSilt fraction for all treatments in 2022 in comparison to 2019 and 2020 (sand: $p = 0.04$, cSilt: $p = 0.021$).

The differences in N-recovery between the treatments are significant in 2018 in the coarse and fine silt/ clay fractions as well as in the BS values (cSilt: $p = 0.007$, fSilt-c: $p = 0.029$, BS: $p = 0.039$). This difference in N-recovery between the treatments in these fractions was also significant in 2020. In the other two years the differences are not significant. In the comparison of just the organic fertilisers (SLA, SLU), there are also significant differences in the N-recovery in 2022. In the fractions fPOM, sand and cSilt, the recovery is significantly higher with SLU in comparison to SLA (fPOM: $p = 0.037$, sand: $p = 0.006$, cSilt: $p = 0.004$).

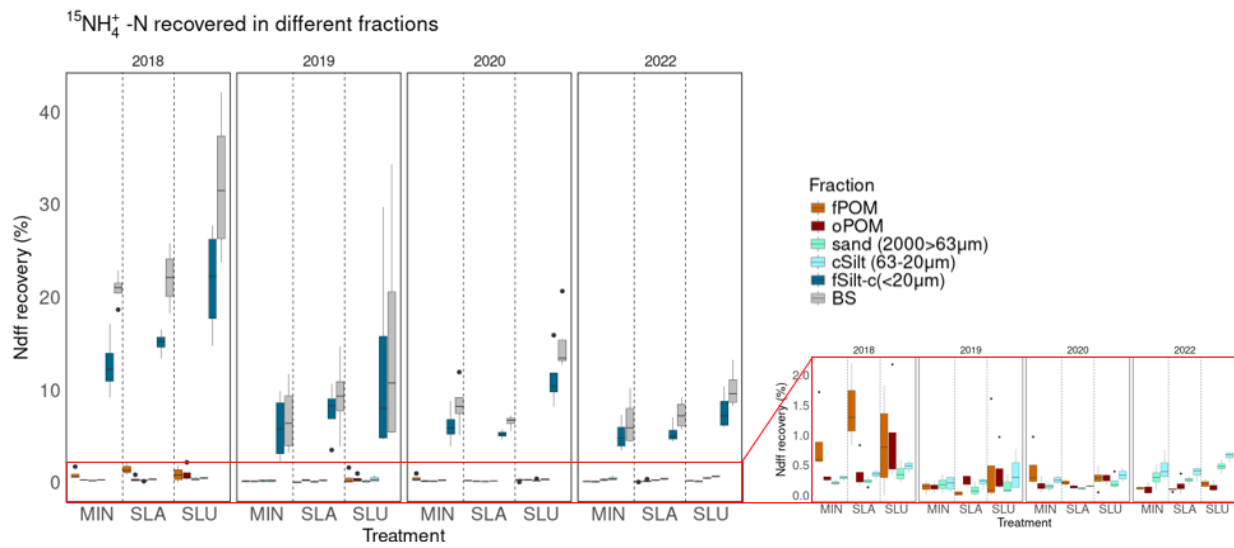


Figure 10: Percentage of the applied NH_4^+ -N that has been recovered in the different fractions. The right is a zoom in of the fractions with lower recovery values.

4. Discussion

4.1 Assessment of method performance

The fractionation method's performance is assessed based on its accuracy, efficiency, and consistency, highlighting its strengths and areas for improvement. The fractionation was successful since mass recovery as well as N_{tot} recovery was over 90% in all samples.

The fPOM and oPOM fractions were at the lower mass limit, making accurate analysis challenging. For improved results in the POM fractions, using a larger BS sample (greater than 10g) would have been beneficial. However, the choice of 10g was made for practical reasons. A larger sample size would not have fit within a single falcon tube, necessitating multiple steps in the density fractionation process (ultrasonication and centrifugation). Conducting these steps two or three times for the same sample would have significantly increased both workload and time requirements.

The density cut-off of 1.8 g/cm³ was selected based on the study by Fuchs et al. (2023). However, this value may not be universally applicable to all soils. Each soil group should first be tested across a range of densities, with the resulting fractions analysed for key parameters relevant to the study. Due to practicability, no tests with different density solutions were performed in the present study. Using a density that is too high could lead to more mineral particles remaining in suspension, thereby increasing the mass of the POM fraction and diluting its C content (Griepentrog & Schmidt, 2013). Since the mass of the POM was very small in the present study, it can be stated that POM was surely not overestimated.

The ultrasonic treatment duration was determined through an initial calibration, which involved estimating the temperature increase over the treatment period. This calibration was performed only once at the start of the laboratory work, and the same duration was subsequently applied to all samples. Ideally, calibration should have been conducted before processing each sample to ensure optimal accuracy. For this study, the same time was used for unity throughout all samples because the samples all derived from the same study site. At the beginning of the laboratory work, different dispersion energies (50 J/mL, 100 J/mL, and 150 J/mL) were tested. However, no significant differences were observed in mass recovery, C content, or N content across the tested energies. Therefore, the same dispersion energy was used as in the study of Fuchs et al. (2023).

In some samples, the total N summed across all fractions is higher than the N_{tot} measured in the BS. This difference may arise because the soil sample is not completely homogeneous. The small portion of soil used for the BS measurement may differ slightly from the portion used for fractionation, leading to minor variations in the results. This also results in a recovery of above 100% for the N_{tot} as visible in Figure 5 in the year 2022 for MIN treatment. These differences are within the range of expected measurement variability. The sum of the N_{tot} values of the fractions lies in between the standard deviation of the BS.

In 2018, the recovery of ¹⁵N was significantly lower compared to previous years (Figure 6). When compared to the recovery of N_{tot} and mass, the percentage of ¹⁵N recovery was also notably lower. This suggests that the losses were likely not caused by the fractionation process in general, as similar losses in N_{tot} and mass would have been observed. It is more probable that the ¹⁵N added in 2018 was still in mineral form, which led to its extraction from the sample

through the SPT solution or the water used to wash the samples after density fractionation. In subsequent years, the amount of N that could be easily extracted decreased as the added ^{15}N became more stabilised. To accurately quantify the extracted ^{15}N , the SPT solution used in the fractionation process would also need to be measured for ^{15}N abundance.

In conclusion, the density fractionation method proved to be a reliable and effective technique for SOM separation. Refinements such as more frequent calibration checks and soil-specific density adjustments could further optimise the results. However, the approach successfully provided meaningful and reproducible insights into soil fraction characteristics.

4.2 Characteristics of fractions

Variations in fractions and across years are already noticeable in the N_{tot} abundance. These fluctuations may significantly impact the interpretation of the distribution of newly applied fertiliser N, particularly the immobilisation and stabilisation processes. Consequently, it is essential to first examine the overall abundance of N_{tot} before addressing the dynamics and transformations of fertiliser N.

The highest concentration of N_{tot} is found in the oPOM fraction, which exceeds that in the fPOM fraction. Consequently, the C/N ratio is lower for oPOM than for fPOM, reflecting the more advanced degradation state of oPOM. As organic matter decomposes, N tends to be retained, while C is lost through the consumption and respiration of microbes. This leads to a reduced C/N ratio in more decomposed fractions. The amount of N in the POM fractions has nevertheless not a high influence on the N_{tot} storage because the pool mass is just very small.

The C/N ratio of MAOM fractions is consistently lower than that of POM fractions. This is likely because POM consists of partially decomposed plant residues, whereas MAOM is composed of more stabilised organic matter, where microbial processing has already led to an even lower C/N ratio. Notably, the fSilt-c fraction exhibited a lower C/N ratio compared to the sand and cSilt fractions, a pattern also observed by Fuchs et al. (2023). They noted, this difference could be attributed to POM material passing through the 63 μm sieve but not the 20 μm sieve.

Overall, the C/N ratios of the POM fractions as well as the sand and cSilt fractions in this study were higher than those reported by Fuchs et al. (2023). The different soil characteristics, as well as different crop rotation, could lead to these higher values. This suggests potential differences in organic matter composition, decomposition dynamics, influencing N availability and stabilisation. The higher C/N ratio observed in this study may have implications for N immobilisation rates, potentially leading to distinct nutrient cycling patterns compared to those in Fuchs et al. (2023). A particularly striking trend was the notably high C/N ratio in 2019. This could be attributed to the presence of cover crop residues remaining on the field during that growing period. Cover crops, especially those with high C content, can contribute to an increased C/N ratio in SOM, as their decomposition releases C-rich material while N remains relatively immobilised (Lucadamo et al., 2022).

In this study, the clay content at the site averaged $18 \pm 1.0\%$ (Bünemann-König & Mayer, 2021). Through the size fractionation in this study, the fSilt-c content was on average

42%±2.8%. Through the sieve size of 20 µm, this percentage is the sum of the clay content and the fine silt. The extent to which soil retains N is closely tied to its clay content, as clay-rich soils provide more binding sites for N compounds (Fuchs et al., 2023). Therefore, across all sampling years, the majority of N_{tot} was found in MAOM fractions, with 87% in the fSilt-c fraction and 93% in the overall MAOM fraction. Overall, MAOM fractions play a key role in stabilising N. In contrast, POM fractions have a high N concentration, their contribution to overall N storage is nevertheless minimal due to their small pool size.

4.3 Nitrogen derived from fertiliser in fractions

As seen in the previous section, the majority of the N is stored in the MAOM pool. The percentage of ¹⁵NH₄⁺ derived N in MAOM fractions is lower than for N_{tot}, but also the applied N was found as a mean of all sampling years and treatments with 72% in the fSilt-c fraction and 76% in the overall MAOM fraction. These values align with findings from other studies, such as Fuchs et al. (2023) and Jilling et al. (2018), indicating a consistent pattern of N stabilisation in soil. Applied ¹⁵N not taken up by plants was either directly incorporated into MAOM or immobilised by soil microorganisms during the first growing period and then incorporated into the MAOM.

The development of Nd_{ff}, particularly between the application in 2018 and 2019 in the fSilt-c fraction, closely correlates with the recovery in the BS (Figure 9). Since the majority of residual ¹⁵N was found in the MAOM fraction, this fraction appears to be the primary source of fertiliser-derived N for plants across all treatments (Fuchs et al., 2023). After the application, ¹⁵NH₄⁺ is adsorbed onto clay minerals and is temporarily bound to them. This bound N can easily be extracted and exchanged and therefore, the amount of ¹⁵NH₄⁺ derived N decreases rapidly in the first year (from 2018 to 2019) in the fSilt-c fraction. As noted by Fuchs et al. (2023), these findings support the idea that plants can access nutrients from MAOM through both direct mechanisms (via root exudates) and indirect mechanisms (via microbial activity) (Jilling et al., 2018).

The amount of Nd_{ff}, particularly in the fPOM fraction but also in oPOM, decreased between 2018 and 2019 (Figure 9). This indicates that the labelled ¹⁵NH₄⁺-N was initially taken up by plants but then remained in the soil as plant residues and dead roots. As the Nd_{ff} (kg/ha) amount decreased over time, it suggests that N from POM was mineralised, making N available for further plant uptake or adsorption onto mineral surfaces. These findings support the first part of the second hypothesis, which proposes that Nd_{ff} is progressively cycled through organic and mineral-associated pools.

A shift of Nd_{ff} from POM to MAOM, as predicted in the second part of the second hypothesis, cannot be confirmed by the data. Over the five-year period, Nd_{ff} in the fSilt-c fraction declined, while Nd_{ff} (kg/ha) in the cSilt and sand fractions increased. However, this increase is primarily due to the rise in mass fraction and N_{tot} rather than an actual shift in labelled N. The data do not show an increase in atom% ¹⁵N excess (Figure 8), indicating that the observed rise in N recovery is simply a result of the multiplication with N_{tot} and the mass fraction rather than an enrichment of labelled N. The higher N_{tot} values may be explained by the fact that, at this

sampling date, the last (unlabeled) fertiliser application had occurred more recently compared to other sampling dates, potentially influencing N dynamics in the soil.

To conclude, the majority of the applied $^{15}\text{NH}_4^+$ was already found in the MAOM fraction in the year of application (2018), emphasising its role in N, adsorption, stabilisation and plant availability. Although the POM fractions have a fast turnover time, the amount of N involved is very small in comparison to the N stored in the MAOM fraction.

4.4 Recovery of NH_4^+ -N derived from MIN, SLU and SLA

The different characteristics of the treatments further influence the recovery of the applied $^{15}\text{NH}_4^+$. This leads to different NUE, losses and finally to a different residual ^{15}N in the year of application as well as the following years.

As explained in section 4.1, more ^{15}N than N_{tot} was lost during fractionation in the first sample year, which suggests that the recently added ^{15}N was still in mineral form and, therefore, extractable (Fuchs et al., 2023). This effect was especially pronounced for MIN, where fractionation losses were higher. This may indicate that the stabilisation rate for MIN is lower than for the other fertiliser treatments, making its N more susceptible to being lost.

A similar trend is observed in the N recovery data (Figure 6), where the residual ^{15}N in the soil is considerably lower for MIN compared to SLU and SLA. The lower recovery in MIN is partially due to the higher NUE by the plants. The higher immobilisation rate in SLU can be attributed to its higher C/N ratio, which promotes microbial N retention. SLU contains a greater amount of labile organic C compounds, which serve as an easily accessible energy source for soil microorganisms (Petersen, 2006). In contrast, MIN provides no additional C, and in SLA, much of the readily decomposable organic C has already been broken down during anaerobic digestion (Webb et al., 2011). As a result, NH_4^+ -N recovery in the soil in 2018 was highest for SLU, followed by SLA and then MIN.

Another factor that could contribute to this result is the lower pH value of SLU, which favours stronger bound types to minerals (Bingham & Cotrufo, 2016). Therefore, the uptake by plants, the accumulation of NH_4^+ in deeper layers and/or the losses have been higher with MIN and SLA than SLU. Data from the same study site indicate that during the first growing period in 2018, plant recovery of the applied $^{15}\text{NH}_4^+$ -N was approximately 34% for MIN and 15% for both SLU and SLA (Bünemann-König & Mayer, 2021, unpublished data). The NUE was particularly low due to the hot and dry weather conditions that likely limited plant uptake (Figure 2). As a result, only 32% (SLA) to 48% (MIN) of the applied $^{15}\text{NH}_4^+$ -N was recovered in either the plants or the topsoil. The remaining N was either stored in the subsoil or lost through processes such as leaching, volatilisation, or denitrification. This also leads to the conclusion that the N loss or shift to the subsoil was highest for SLA because the NUE of the plants in 2018 was not as high as for MIN, and the residual effect in the topsoil was not as high as for SLU. This could nevertheless be because of the overall low NUE in this year. The high pH in SLA (7.9 compared to 7.1), increases the risk of NH_3 volatilisation (Webb et al., 2011), which further supports the conclusion that N losses were high in SLA.

Furthermore, the desorption rate of the temporarily adsorbed $^{15}\text{NH}_4^+$ -N on minerals is slower for SLU than for SLA and MIN, and the recovery of $^{15}\text{NH}_4^+$ -N was higher in 2018. This trend continued into 2019, where the residual effect for SLU was still higher than for SLA and MIN. Nevertheless, the N recovery is for all treatments under 14% in the sum of the percentage of the single fractions. For all treatments, the N recovery in the topsoil halved in 2019 in comparison to the values in 2018. This means that the majority of the previously adsorbed $^{15}\text{NH}_4^+$ on minerals was released within this period. This confirms the first hypothesis that the amount of fertiliser $^{15}\text{NH}_4^+$ -N decreased rapidly in the first year.

In 2020, a noticeable increase in residual N was observed across all fractions for MIN. This increase may be attributed to the uptake of $^{15}\text{NH}_4^+$ -derived N from the subsoil, followed by its subsequent decomposition and stabilisation in the topsoil. The movement of N from deeper soil layers could indicate delayed N availability, potentially influenced by factors such as soil texture, microbial activity, or seasonal variations in mineralisation and leaching dynamics (Haberle et al., 2006). To fully understand the mechanisms behind this increase in N recovery for MIN, further research focusing on subsoil N dynamics is essential. Furthermore, it is unclear why this increase could just be with MIN and not also with the SLA and SLU.

In general, after 2019 the difference in N recovery between the treatments remains the same (SLU>SLA>MIN) and the percentage in all treatments remains in sum of all fractions nearly stable (Mean value MIN: 6.57%, SLA: 7.73% and SLU: 13.50% of applied NH_4^+ -N) (Figure 10). This implies that the remaining Ndff is stabilised and is not (re)mineralised easily. Consequently, a very slow decrease in residual N can be expected in the subsequent years. This slow decline is likely due to its increasing integration into more resistant forms of SOM, which are less susceptible to microbial decomposition (Sørensen & Amato, 2002). This observation supports the second part of the first hypothesis, suggesting that the long-term availability of N is reduced as it becomes part of stable organic matter fractions.

The significantly higher residual effect of SLU in comparison to SLA after five years further validates the third hypothesis. This finding suggests that SLU treatment has a more lasting impact on N retention and stabilisation in the soil compared to SLA. In contrast, the difference between SLA and MIN in terms of N recovery is no longer statistically significant after five years.

The recovery and stabilisation of applied $^{15}\text{NH}_4^+$ -N are influenced by the characteristics of the treatments. SLU consistently shows the highest residual effect in the SOM fractions, highlighting its capacity for N retention and stabilisation over time. In contrast, SLA and MIN demonstrate lower recovery of NH_4^+ in the soil. In all treatments, the data highlighted the critical role of the fSilt-c fraction in N exchange, particularly during the year of application (2018) and the subsequent year (2019).

5. Conclusion

POM and MAOM represent distinct pools for N. While POM serves as a rapidly cycling pool, MAOM provides stabilisation over a longer period. Applied N as ammonium can either temporarily get adsorbed on the clay minerals and then, with a relatively fast turnover time, be exchanged again. It can also be stabilised over a longer time, through plant residues or immobilisation and then as organic N bound and protected by minerals from degradation.

The results showed that POM fractions have the highest N concentration but minimal contribution to overall N storage due to their small pool size. In contrast, MAOM fractions dominate N storage and show a fast incorporation of N. Also, for the applied N the MAOM, especially the fSilt-c fraction, was the most important storage. A shift of Ndff from POM to MAOM could not be confirmed, the observed increases in N recovery were linked to changes in Ntot and mass fractions rather than labelled N enrichment.

The recovery and stabilisation of $^{15}\text{NH}_4^+$ -derived N is influenced by the treatment characteristics. SLU consistently exhibited the highest residual N effect in all fractions, emphasising its capacity for long-term retention and stabilisation compared to SLA and MIN. Five years after the application of labelled fertiliser, a higher percentage of $^{15}\text{NH}_4^+$ -N was recovered from SLU than from SLA. This also means that this residual effect has to be considered in the application of fertilisers to optimise the synchronicity in the availability and the N needs of plants. Across all treatments, N recovery in the topsoil decreased after 2018, highlighting the rapid release of poorly stabilised N within the first year. After 2019, the applied N stabilised within the fractions, and changes were rather small in the subsequent years. In future the recovered N after 5 years in the soil should be addressed to gain a better understanding of how the N content in the soil accumulates over even longer time periods, if after every fertiliser application, a small percentage of N remains in the soil.

The results of this study are specific for one experimental field with a specific climate and soil characteristics. In a further study step, the results of this thesis could be compared to the plant uptake and the residual N in the subsoil (>20cm) in these years to gain a better understanding of the N dynamics and the effective losses of the applied N for each fertiliser.

The findings through the fractionation contribute to a better understanding of the N dynamics. This is a further step to compromise the NUE, yield and environmental impacts to a more sustainable agriculture.

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8. Appendix



Figure A11: Drone image of the experimental field in Wallbach. The colours show the different fertilised plots. The labelled plots were located within these plots.

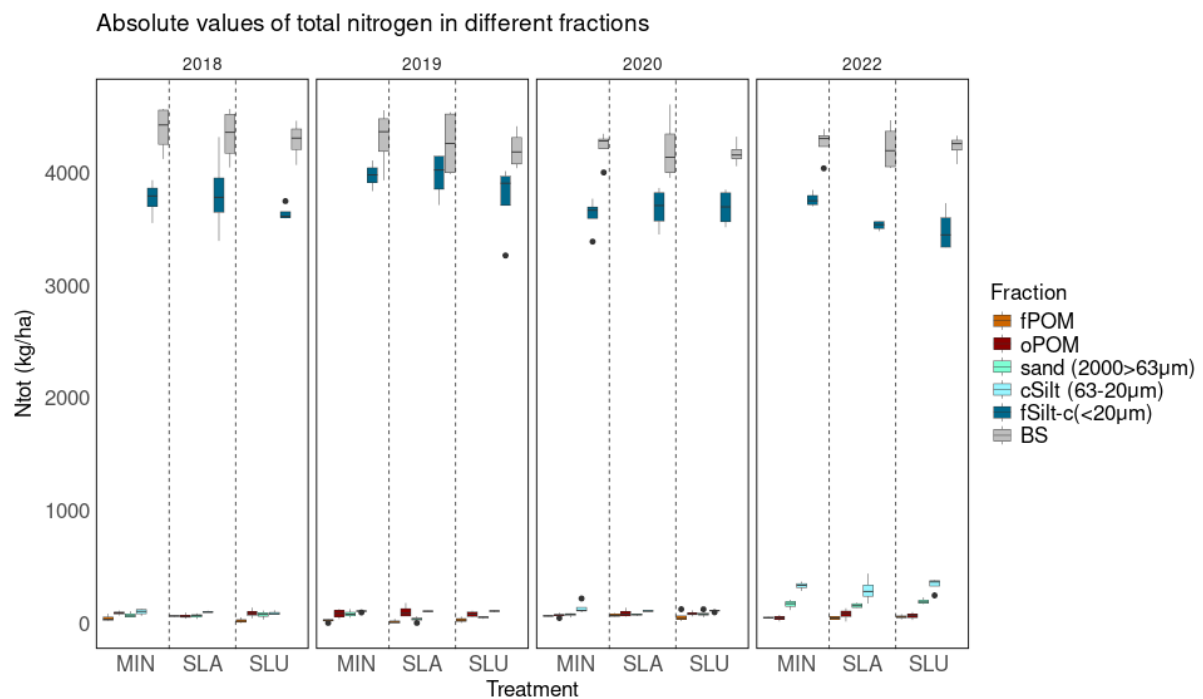


Figure A12: Additional plot of the Ntot in the different fractions in absolute values (kg/ha). In the plot, the POM fractions show a minor contribution to the Ntot mass, indicating their limited role in long-term stabilisation.

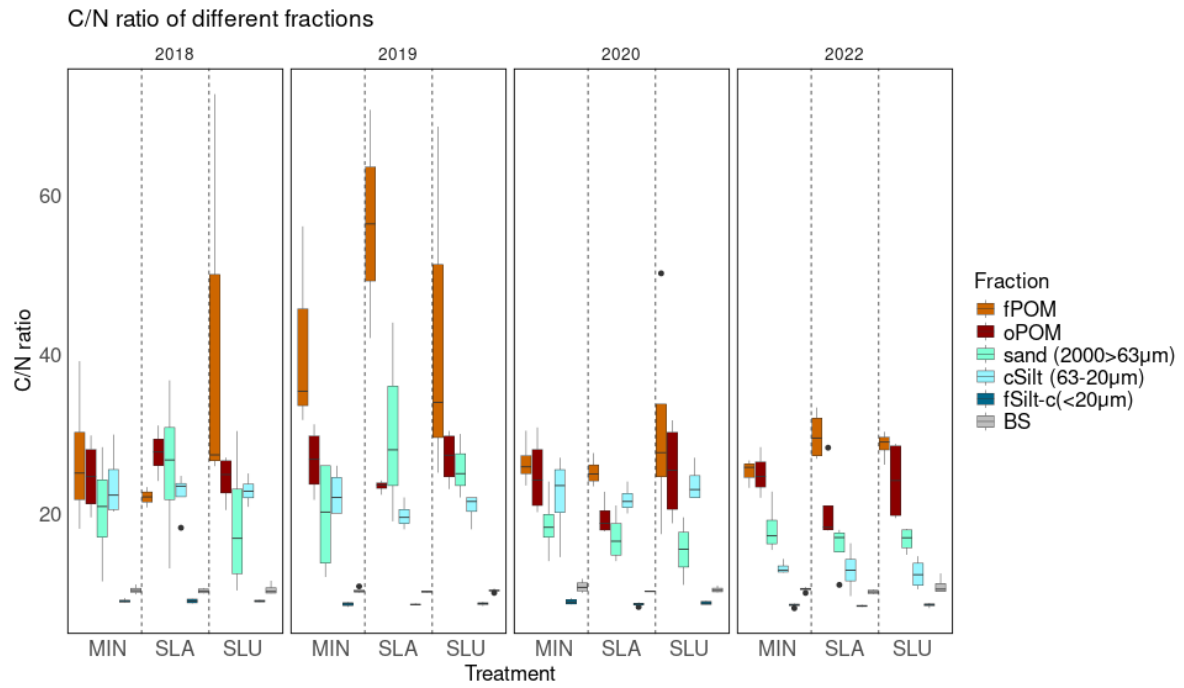


Figure A13: Boxplot of the C/N ratio values of the different fractions for the treatments and years.

Table A4: Tables with the measured and calculated values (Mass of the fractions after fractionation in grams, Ntot in percent, atomic percent ¹⁵N excess in the fractions, N derived from fertiliser in percent, N derived from fertiliser in absolute values (kg/ha) and recovery of the applied labelled fertiliser in the fractions). The values are the means of the 4 replicates with the standard deviation. The letters in the stat. columns shows the statistical difference between the fractions, treatments and years. The lowercase letters indicate the significant difference of this value in comparison to the other years in that fraction and treatment. The capital letters indicate the difference between fractions and treatments. If there are two capital letters, the first indicates the difference or similarity between the fractions and the second between the treatments.

Mass (g)	Fraction	2018			2019			2020			2022		
		Mean±SD	Stat.	SLU	Mean±SD	Stat.	SLU	Mean±SD	Stat.	SLU	Mean±SD	Stat.	SLU
Ntot (%)	IPOM	0.018 ± 0.016	A	0.028 ± 0.006	BA	0.01 ± 0.005	A	0.009 ± 0.005	A	0.013 ± 0.007	A	0.027 ± 0.01	A
	oPOM	0.016 ± 0.005	A	0.014 ± 0.004	A	0.026 ± 0.018	A	0.018 ± 0.008	A	0.016 ± 0.006	A	0.019 ± 0.007	A
	sand (2000-63µm)	1.829 ± 0.735	C	1.866 ± 0.703	C	1.876 ± 0.533	C	1.878 ± 0.675	hC	1.868 ± 0.534	C	1.987 ± 0.77	hC
	cSilt (63-20µm)	3.388 ± 0.245	D	3.652 ± 0.368	D	3.619 ± 0.238	D	3.615 ± 0.253	D	3.606 ± 0.303	D	3.708 ± 0.312	D
	fSilt-c(<20µm)	4.384 ± 0.427	F	4.216 ± 0.28	F	4.399 ± 0.334	E	4.359 ± 0.459	E	4.101 ± 0.211	E	4.089 ± 0.321	D
Atom% ¹⁵ N excess	BS	10.02 ± 0.026	F	10.009 ± 0.007	F	10.011 ± 0.004	F	10.006 ± 0.002	F	10.006 ± 0.002	F	10.003 ± 0.003	F
Ndiff (%)	IPOM	0.99 ± 0.22	B	0.81 ± 0.31	B	0.63 ± 0.52	B	0.37 ± 0.38	B	0.58 ± 0.42	B	0.97 ± 0.22	B
	oPOM	1.91 ± 0.21	C	1.54 ± 0.29	C	1.62 ± 0.25	C	1.48 ± 0.24	C	1.55 ± 0.27	C	1.5 ± 0.14	C
	sand (2000-63µm)	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A
	cSilt (63-20µm)	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A	0.01 ± 0.01	A
	fSilt-c(<20µm)	0.3 ± 0.03	D	0.31 ± 0.04	D	0.3 ± 0.01	D	0.32 ± 0.02	B	0.32 ± 0.02	B	0.35 ± 0.01	hD
Ndiff (kg/ha)	BS	0.15 ± 0.01	E	0.15 ± 0.01	E	0.15 ± 0.01	E	0.15 ± 0.02	E	0.15 ± 0.01	E	0.15 ± 0.01	E
Ndiff (kg/ha)	IPOM	0.221 ± 0.056	hB	0.139 ± 0.05	B	0.196 ± 0.065	B	0.073 ± 0.028	B	0.075 ± 0.068	B	0.072 ± 0.058	B
	oPOM	0.034 ± 0.01	A	0.023 ± 0.015	A	0.048 ± 0.023	A	0.023 ± 0.017	A	0.031 ± 0.037	A	0.022 ± 0.01	A
	sand (2000-63µm)	0.032 ± 0.007	A	0.022 ± 0.006	A	0.028 ± 0.008	A	0.024 ± 0.02	A	0.019 ± 0.006	A	0.009 ± 0.001	AD
	cSilt (63-20µm)	0.031 ± 0.006	A	0.021 ± 0.003	A	0.029 ± 0.009	A	0.02 ± 0.014	A	0.019 ± 0.017	A	0.021 ± 0.007	A
	fSilt-c(<20µm)	0.035 ± 0.009	hA	0.024 ± 0.003	hA	0.03 ± 0.009	A	0.015 ± 0.009	A	0.017 ± 0.015	A	0.018 ± 0.006	A
Ndiff (kg/ha)	BS	0.049 ± 0.006	hA	0.031 ± 0.003	hA	0.038 ± 0.01	hA	0.017 ± 0.01	A	0.013 ± 0.006	A	0.02 ± 0.007	A
Ndiff (kg/ha)	IPOM	3 ± 0.76	B	1.88 ± 0.68	B	2.66 ± 0.88	B	0.99 ± 0.39	A	0.37 ± 0.16	A	0.98 ± 0.79	A
	oPOM	0.46 ± 0.13	A	0.44 ± 0.2	A	0.65 ± 0.32	A	0.31 ± 0.23	A	0.25 ± 0.15	A	0.3 ± 0.14	A
	sand (2000-63µm)	0.43 ± 0.1	A	0.31 ± 0.08	A	0.38 ± 0.11	A	0.33 ± 0.27	A	0.21 ± 0.1	A	0.22 ± 0.22	AF
	cSilt (63-20µm)	0.47 ± 0.12	A	0.33 ± 0.04	A	0.41 ± 0.12	A	0.21 ± 0.13	A	0.16 ± 0.06	A	0.24 ± 0.08	A
	fSilt-c(<20µm)	0.43 ± 0.08	eA	0.29 ± 0.04	eA	0.39 ± 0.12	A	0.27 ± 0.19	A	0.17 ± 0.07	A	0.23 ± 0.23	A
Ndiff (kg/ha)	BS	0.67 ± 0.08	CA	0.42 ± 0.04	A	0.52 ± 0.14	A	0.23 ± 0.13	A	0.18 ± 0.08	A	0.28 ± 0.09	A
Ndiff (kg/ha)	IPOM	1.2 ± 0.8	B	1.18 ± 0.56	B	0.58 ± 0.56	AB	0.19 ± 0.14	A	0.02 ± 0.03	BA	0.61 ± 0.51	A
	oPOM	0.39 ± 0.05	A	0.3 ± 0.25	A	0.62 ± 0.58	A	0.19 ± 0.11	A	0.25 ± 0.07	A	0.23 ± 0.16	A
	sand (2000-63µm)	0.29 ± 0.06	A	0.19 ± 0.06	A	0.26 ± 0.11	A	0.26 ± 0.18	A	0.07 ± 0.08	A	0.19 ± 0.09	A
	cSilt (63-20µm)	0.4 ± 0.05	A	0.28 ± 0.05	A	0.33 ± 0.06	A	0.27 ± 0.18	A	0.17 ± 0.07	A	0.36 ± 0.09	A
	fSilt-c(<20µm)	17.76 ± 4.65	hE	12.31 ± 1.06	hE	14.85 ± 4.14	ED	8.31 ± 5.14	E	6.28 ± 2.45	E	8.6 ± 8.07	E
Ndiff recovery (%)	BS	29.26 ± 2.41	hE	18.02 ± 2.69	hE	22.01 ± 5.7	E	9.71 ± 5.5	E	7.59 ± 3.62	E	11.71 ± 3.9	E
Ndiff recovery (%)	IPOM	0.86 ± 0.57	hA	1.44 ± 0.69	BA	0.86 ± 0.82	A	0.13 ± 0.1	A	0.02 ± 0.03	BA	0.44 ± 0.36	A
	oPOM	0.28 ± 0.04	A	0.37 ± 0.31	A	0.91 ± 0.85	A	0.18 ± 0.08	A	0.25 ± 0.09	A	0.16 ± 0.11	A
	sand (2000-63µm)	0.21 ± 0.04	DA	0.23 ± 0.07	A	0.38 ± 0.16	A	0.09 ± 0.1	A	0.06 ± 0.17	A	0.14 ± 0.06	A
	cSilt (63-20µm)	0.29 ± 0.04	A	0.35 ± 0.06	A	0.48 ± 0.09	AF	0.19 ± 0.13	A	0.37 ± 0.31	A	0.26 ± 0.06	A
	fSilt-c(<20µm)	12.68 ± 3.32	hF	15.08 ± 1.3	hF	21.75 ± 6.07	hF	5.93 ± 3.67	F	7.67 ± 3	F	12.59 ± 11.82	F
Ndiff recovery (%)	BS	20.9 ± 1.72	hG	22.09 ± 3.29	hG	32.23 ± 8.34	hF	6.93 ± 3.93	F	9.5 ± 4.3	F	15.28 ± 13.64	F

Personal declaration:

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

Place, date: Zürich, 24.04.2025

Signature:

A handwritten signature in blue ink, appearing to be 'F. Alin'.