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old N ?

# Old organic matter in temperate forest soils is very nitrogen-rich

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**Abstract.** Soil organic matter (SOM) is important for Earth's climate regulation and primary production. Here we tested the hypothesis that old SOM fractions are rich in organic nitrogen given the high affinity of organic nitrogen compounds to interact with mineral surfaces. For this purpose, we studied temperate broadleaf and coniferous forest soils located in Sweden. We used ramped thermal fractionation to obtain persistent SOM fractions, and we evaluated the persistence using $^{14}C$. The results show that old SOM fractions in the temperate forest soils are very nitrogen-rich, indicating that organic nitrogen compounds persist for a long time in soil. A larger proportion of the total organic nitrogen than of the total organic carbon was found in the old SOM fractions, suggesting that a large part of the organic nitrogen pool decomposes on average more slowly than the pool of nitrogen-free organic compounds. The size of the old SOM fractions was correlated with the soil clay content but did not differ significantly between broadleaf and coniferous forests, suggesting that association with minerals rather than forest type or organic matter quality affect the persistence of SOM. This study has important implications for element cycling as it demonstrates that nitrogen in temperate forests persists for a long period in SOM.

## 1. Introduction

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Soil organic matter (SOM) plays an important role in the Earth system ~~since~~ it contains large stocks of carbon and nitrogen (N) that are important for Earth's climate regulation and soil fertility (Batjes, 2014; Friedlingstein et al., 2025). Soil organic N (ON) consists mainly of proteinaceous material, peptides, heterocyclic N compounds, amino acids, and amino sugars (Knicker, 2004). Hence, many ON compounds have functional groups that have a high affinity to adsorb to mineral surfaces in soils, such as amino and carboxyl groups (Yu et al., 2013; Spohn et al., 2024). In addition, many proteins have a high affinity to bind to hydrophobic surfaces on minerals due to the hydrophobic properties of some of their domains (Haynes and Norde, 1994; Yu et al., 2013). The sorption of SOM to mineral surfaces is one of the most important mechanisms that stabilize SOM against decomposition (Kleber et al., 2015; Wiesmeier et al., 2019). Thus, it can be hypothesized that ON decomposes slowly in soil, more slowly than N-free organic compounds, and that old SOM fractions are particularly N-rich (Spohn et al., 2024). ?

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While organo-mineral interactions have been studied intensively ~~during the last~~ years, the role of organic matter quality for SOM sequestration is still not well understood (Wiesmeier et al., 2019). Organic matter quality refers to the chemical composition and the stoichiometry of organic matter. Broadleaf and coniferous forest soils differ substantially in organic matter



low C/N
high C/N
} C, N not independent pools

https://doi.org/10.5194/egusphere-2026-3238
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quality (Cools et al., 2014; Högberg et al., 2021), yet it is still not well understood if this difference in organic matter quality affects SOM sequestration and the sizes of old organic carbon and ON fractions in forest soils.

Recent studies concluded that a ramped thermal treatment makes it possible to isolate carbon fractions that have persisted in soil for different amounts of time. Specifically, these studies reported that the $\Delta^{14}C$ of the $CO_2$ that is emitted from a soil sample during a ramped thermal treatment, decreases with increasing temperature (Sanderman and Grandy, 2020; Stoner et al., 2023). The $\Delta^{14}C$ expresses the $^{14}C$:$^{12}C$ ratio, and it decreases with increasing carbon age. In the present study, a ramped thermal treatment was used for the first time to isolate old SOM fractions as solids for subsequent soil chemical and isotope analyses.

We tested the hypothesis that old SOM fractions are rich in ON irrespective of forest type (broadleaf versus coniferous). To test this hypothesis, we chose old-growth forests that are very similar in terms of climate, soil genesis, and humus form but differ in terms of forest type (broadleaf or coniferous) and soil texture. We used ramped thermal fractionation (Sanderman and Grandy, 2020; Stoner et al., 2023) to obtain persistent SOM fractions, and we evaluated the persistence of the SOM using $^{14}C$.

## 2. Materials and Methods

### *2.1 Sites and soils*

For this study, we chose old-growth forests that are very similar in terms of climate, soil genesis, and humus form but differ in terms of forest type (broadleaf or coniferous) and soil texture. The forest sites are located in southern Sweden (Fig. 1) and were selected using the database of the Swedish Forest Soil Inventory (for a detailed description of the inventory see Spohn et al., 2025).

All soils were chosen to be Regosols and to have the humus form Mull. We selected only Regosols for this study to compare soils with similar genesis and avoid soils with strong eluviation of SOM in the topsoil, which is typical for Podzols. Regosol is the second most abundant soil order in Swedish forests after Podzol (Spohn and Stendahl, 2024). We selected soils with the humus form Mull because these soils do not have an almost mineral-free organic layer on top of the mineral soil, like for example soils with the humus form mor or moder. Instead, the top of these soils is formed by an A horizon, of which we sampled the uppermost 10 cm for this study (see below).

Furthermore, we selected sites with a stand age ~~larger~~ greater than 55 years. In addition, we selected sites that have only broadleaf trees or only coniferous trees, and we excluded sites that have mixes of the two to compare distinct forest types. These sites have very likely never been clear cut and there is likely a long continuity in tree species beyond the current stand age, which means that there has been a long continuity in the quality of the organic matter inputs to the soils. The coniferous forests have the tree species Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.), while the broadleaf forests have the tree species European beech (*Fagus sylvatica* L.), European aspen (*Populus tremula* L.) and English oak (*Quercus robur* L.). We excluded sites located above 60°N to restrict the analysis to sites that have a similar climate. In addition, we selected sites with a soil pH of the A horizon below 6.2 to exclude carbonate-containing soils. Furthermore, we selected sites with contrasting soil texture.

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


We found 12 broadleaf sites and 10 coniferous sites in the database of the Swedish Forest Soil Inventory that matched our criteria. These forest sites are scattered all over southern Sweden (Fig. 1). Their mean annual temperature varies between 5.5 and 8.3°C, and their mean annual precipitation varies between 527 and 769 mm. The mean latitudes of the 12 broadleaf and 10 coniferous forests are 57.8 (± 1.4) and 58.7 (± 1.0), respectively, while the mean longitudes are 14.6 (± 1.7) and 16.3 (± 2.3), respectively (Table 1). The mean stand ages of the broadleaf and coniferous forests are 89 (± 16) and 87 (± 43) years, respectively. The mean stem diameters are 377 (± 154) and 342 (± 45) mm, respectively. The mean soil pH values (measured in water) are 4.9 (± 0.5) and 5.1 (± 0.8), respectively, while the mean clay contents in the A horizon are 13.7 (± 11.0) and 13.4 (± 8.5) %, respectively (see Table 1). The upper 10 cm of the A horizon of the 22 soils were sampled based on a small soil profile using a shovel between 2014 and 2020.

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### *2.2 Soil preparation, chemical and physical analyses*

separate characterization from analysis

All soil samples were dried to constant weight at 35°C. The dry soil samples were homogenized and sieved (< 2 mm) and roots were removed.

We conducted a ramped thermal fractionation of the soils to isolate old SOM fractions. We chose the temperatures for the old SOM fractions to be 325°C and 400°C according to the results of previous studies (Sanderman and Grandy, 2020; Stoner et al., 2023). We obtained two SOM fractions that differ in thermostability to compare the thermostability of TOC and TON at different temperatures.

Prior to the fractionation, all soils were ground using a ball mill. For the fractionation, 1.6 g of soil were exposed to a ramped temperature treatment in a furnace ventilated with ambient air (Nabertherm with temperature controller C19) with an increase in temperature of 5°C per minute until either 325°C or 400°C were reached.

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The total organic carbon (TOC) and total nitrogen concentrations of the bulk soils and soil fractions were analyzed using an elemental analyzer (TruMac CN, LECO). We also determined the inorganic N of all soils and soil fractions to calculate the total organic nitrogen (TON) as the difference between the total N and the total inorganic N. Inorganic N was extracted in 2.0 M KCl and determined using an autoanalyzer (Seal Analytical). In the thermostable SOM fractions, no inorganic N was detected. The detection limit of the inorganic N quantification is 0.05 mg N $l^{-1}$, which corresponds to 0.0004 mg N $g^{-1}$ soil. We calculated the molar TOC:TON ratio, based on the moles of organic carbon and organic nitrogen. The soil pH was determined in water (at a soil:water ratio of 1:2.5 w/v) using a Pt electrode (Aquatrode Plus Pt1000, Metrohm).

The soil texture was determined by vis-NIR using an ASD FieldSpec Pro FR scanning instrument (Malvern Panalytical Ltd, Malvern, UK) following Wetterlind et al. (2022). All samples were measured with three replicates. The clay content of the samples was estimated using a memory-based learning method that was trained with 7617 observations and validated with 3806 observations of Swedish soils.

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


### *2.3 Soil isotope analysis*

The $\Delta^{14}C$ of the bulk soils and soil fractions was analyzed using a MIni CArbon DAting System (MICADAS, Ionplus, Dietikon, Switzerland) at the Max Planck Institute for Biogeochemistry in Jena, Germany. The radiocarbon ratio is reported as $\Delta^{14}C$ in per mille [‰], which is the $^{14}C$:$^{12}C$ ratio of the sample with respect to the $^{14}C$:$^{12}C$ ratio of the standard (oxalic acid standard SRM-4990C; Steinhof et al., 2017) including the normalization for $\delta^{13}C$ (fractionation correction) and the correction for the decay between 1950 and the measurement time (Stuiver and Polach, 1977).

Positive $\Delta^{14}C$ values indicate incorporation of bomb-radiocarbon produced by nuclear-bomb tests in the 1950s and 1960s. Negative values indicate that carbon entered the soil before the 1950s and enough time has passed for substantial radioactive decay to occur. The lower the $\Delta^{14}C$ values, the longer the carbon persisted on average since the time of fixation from the atmosphere. The soil samples were collected during a time when the $\Delta^{14}C$ of the atmosphere was still positive. The $\Delta^{14}C$ of the atmosphere reached 0 ‰ in the year 2020 in the Northern Hemisphere Zone 1 (Hua et al., 2021). Since the samples were taken during a relatively short time, we did not correct for potential temporal differences caused by the temporal change in atmospheric $\Delta^{14}C$, as is common practice in studies on soil radiocarbon (e.g., von Fromm et al. 2024).

### *2.4 Statistical analysis*

We conducted regression analyses and student's t-test, where we considered $p < 0.05$ to indicate a statistically significant difference. A map was created using the R package tmap (version 4.0). All data analyses were conducted using R (version 4.4.1, R Core Team, 2021).

## 3. Results

The median $\Delta^{14}C$ of the SOM was 77.8‰, while the median $\Delta^{14}C$ of the SOM fractions that were thermostable at 325°C and 400°C was 48.5‰ and 9.5‰, respectively (Fig. 2A). The median molar TOC:TON ratio of the bulk soil was 19.9, while the median molar TOC:TON ratio of the SOM fractions thermostable at 325°C and 400°C was 7.5 and 4.8, respectively (Fig. 2B). On average, only 33% and 10% of the TOC were part of the SOM pools thermostable at 325°C or 400°C, respectively (Fig. 2C). In contrast, 82 and 38% of the soil TON were present in the SOM pools thermostable at 325°C or 400°C, respectively (Fig. 2D). The $\Delta^{14}C$ as well as the TOC and TON contents in the soils and in the fractions thermostable at 325°C or 400° did not differ significantly between broadleaf and coniferous forests ($P > 0.05$; Fig. 2A, C, and D). Furthermore, the TOC:TON ratio did not differ significantly between broadleaf and coniferous forests in the fraction stable at 400°C, but it was significantly higher in the coniferous forests than in broadleaf forests in the bulk soil and in the 325°C fraction (Fig. 2B).

The percentages of TOC and TON that remained after the 325°C and 400°C treatments were positively correlated with the soil clay content (for TON the $R^2$ were 0.34 and 0.49, respectively; Fig. 3). The adjusted $R^2$ of the regressions of the percentages of TOC (but not TON) in the two old SOM fractions increased when pH was included as a second predictor alongside clay (to 0.49 and 0.50 for the 325°C and 400°C fraction, respectively, both $p < 0.001$; Table 2).

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


## 4. Discussion

Our results support the hypothesis that old SOM fractions are rich in ON, irrespective of forest type, and they suggest that the soil clay content contributes to the stabilization of old ON.

We found large differences in $\Delta^{14}C$ between the bulk soil and the two thermostable SOM fractions, demonstrating that SOM in the thermostable fractions is on average much older than the total SOM. This result is in accordance with previous studies, reporting that ramped thermal fractionation can be used to isolate SOM fractions of different ages (Sanderman and Grandy, 2020; Stoner et al., 2023). The reason why old SOM is on average more thermostable than young SOM is likely that the rate of organic matter oxidation depends on activation energy, independently of whether the oxidation is facilitated by very high temperatures or by enzymes. The increase in temperature during the ramped thermal fractionation facilitates the oxidation of SOM because it adds energy to the soil which allows the organic compounds to overcome the energy barrier formed by the activation energy. In contrast, organic matter decomposition in soils is mainly catalyzed by enzymes that lower the activation energy. Yet, the absolute decrease in activation energy that enzymes cause is similar across different classes of enzymes which catalyze different reactions that have very different activation energies (Sousa et al., 2020). Thus, importantly, the relative differences in activation energy among different types of reactions are only little affected by enzymatic catalysis. This means that even in soils, where decomposition is enzymatically catalyzed, reactions that have a higher activation energy have a lower probability to occur, similar as in the thermal decomposition. The activation energy of the oxidation of adsorbed organic matter includes the energy required for the desorption of the adsorbed organic matter (Williams and Plante, 2018). Our finding that the age of SOM increases with increasing thermostability of SOM suggests that activation energy is a key factor that affects persistence of organic matter in soils (Williams and Plante, 2018).

The old SOM pool stable at 400°C had a very low and highly constrained TOC:TON ratio in the range of the TOC:TON ratio of proteins. The reason why the two old SOM pools were very N-rich is most likely that ON is rich in functional groups that allow ON compounds to adsorb to mineral surfaces and interact with other charged compounds (Yu et al., 2013; Spohn et al., 2024). The high affinity of ON compounds to adsorb to mineral surfaces likely stabilizes ON against decomposition, as indicated by studies reporting that amino acids decompose more slowly than other organic compounds in soil (Miltner et al., 2009) and that minerals restrict protein decomposition and amino acid uptake by microorganisms (Chevallier et al. 2003; Vieublé Gonod et al., 2006; Hunter et al., 2016). The preferential adsorption of ON compounds, compared to many N-free compounds, has been hypothesized to lead to a slow decomposition of soil ON (Spohn, 2024). This is supported by our results showing that old N-rich SOM fractions are very persistent.

We found no significant difference between broadleaf and coniferous forest soils with respect to N and C contents as well as $\Delta^{14}C$ of the two old SOM pools. These results suggest that differences in organic matter quality among broadleaf and coniferous forests (Cools et al., 2014; Högberg et al., 2021) do not significantly affect long-term SOM sequestration. Instead, we found that the percentages of TOC, and especially TON in the old SOM fractions were significantly correlated with the soil clay content, indicating that clay makes SOM persist in soils. Clay likely increases the percentage of OM that has a high affinity to adsorb to mineral surfaces because it slows down the decomposition of this adsorbed OM. The finding that the adjusted $R^2$ of

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


the regressions of clay and the percentage of TOC in the old SOM fractions increased when pH was included as a second predictor suggests that pH also affects stabilization of TOC. The reason for this could be that pH affects soil microbial activity, and hence organic carbon decomposition (Malik et al., 2018) This finding is in accordance with a recent study showing that soil TOC content varies more strongly with pH than the soil N content, while the soil N content varies more strongly than the TOC content with texture in temperate forest soils (Spohn and Stendahl, 2024).

Our results confirm that ramped thermal fractionation is a powerful approach to isolate SOM fractions differing in $\Delta^{14}C$ and hence in persistence (Sanderman and Grandy, 2020; Stoner et al., 2023). Previous studies used ramped thermal fractionation to quantify the $\Delta^{14}C$ of the $CO_2$ that is emitted from the soil during the thermal treatment (Sanderman and Grandy, 2020; Stoner et al., 2023). In contrast, the present study demonstrates that the approach can also be used to isolate old SOM fractions (in solid form) that strongly differ from the bulk soil in $\Delta^{14}C$, allowing for additional chemical analyses of the old SOM. The differences in $\Delta^{14}C$ between the bulk soil and the two thermostable fractions found here were much larger than the differences in $\Delta^{14}C$ between the bulk soil and the mineral-associated fraction obtained by traditional fractionation techniques, such as density fractionation (e.g., Baisden et al., 2002; McFarlane et al., 2013; Lyband et al., 2017), confirming the high potential of ramped thermal fractionation to isolate persistent SOM fractions. In addition, the C:N ratio of the thermostable fractions was strongly constrained and much lower than the C:N ratio typically observed in the mineral-associated SOM fraction based on traditional fractionation methods (Baisden et al., 2002; McFarlane et al., 2013; Lyband et al., 2017), suggesting that thermal fractionation allows for isolation of SOM that differs in terms of stoichiometry and turnover from other SOM pools.

Taken together, in the present study, a ramped thermal treatment was used for the first time to isolate old SOM fractions as solids to perform isotope and element analyses on these fractions. Our results demonstrate that old SOM is very N-rich and they indicate that the percentage of very old SOM is mainly controlled by the soil clay content but not by forest type. A larger proportion of TON than of TOC was found in the old SOM pools, indicating that large part of the soil TON pool decomposes more slowly than N-free organic compounds in these soils. Our study has important implications for element cycling since it demonstrates that N in temperate forests persists for a long period in SOM.

**Table 1** Mean geographic, stand, and soil properties of the 22 broadleaf and coniferous forests located southern Sweden. The numbers depict means (± standard deviations) of the 12 broadleaf and 10 coniferous forests.

| Forest type | Latitude (°N) | Longitude (°E) | Stand age (years) | Average stem diameter (mm) | Standing wood volume coniferous ($m^3$ $ha^{-1}$) | Standing wood volume broadleaf ($m^3$ $ha^{-1}$) | Soil clay content (%) | Soil pH (in $H_2O$) |
|---|---|---|---|---|---|---|---|---|
| Broad-leaf (N = 12) | 57.8 (± 1.4) | 14.5 (± 1.7) | 89.0 (± 15.5) | 376.5 | 0.0 (± 0.0) | 299.6 (± 193.9) | 13.7 (± 11.0) | 4.9 (± 0.5) |
| Coni-ferous (N = 10) | 58.7 (± 1.0) | 16.3 (±1.4) | 87.3 (± 42.7) | 341.8 | 401.0 (± 297.3) | 0.0 (± 0.0) | 13.3 (± 8.5) | 5.1 (± 0.9) |

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


200 **Table 2** Summary of multiple regressions of percentages of TOC and TON remaining after the thermal treatment up to 325°C and 400°C as a function of soil clay content and pH.

| Dependent variable | Independent variables | P | Adj. $R^2$ |
|---|---|---|---|
| ~~Fraction~~ prop. of TOC after 325°C (%) | Clay content + pH | < 0.001 | 0.49 |
| Fraction of TOC after 400°C (%) | Clay content + pH | < 0.001 | 0.50 |
| Fraction of TON after 325°C (%) | Clay content + pH | < 0.001 | 0.28 |
| Fraction of TON after 400°C (%) | Clay content + pH | < 0.001 | 0.45 |

model selection vs. clay alone? } table vs. graph?

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


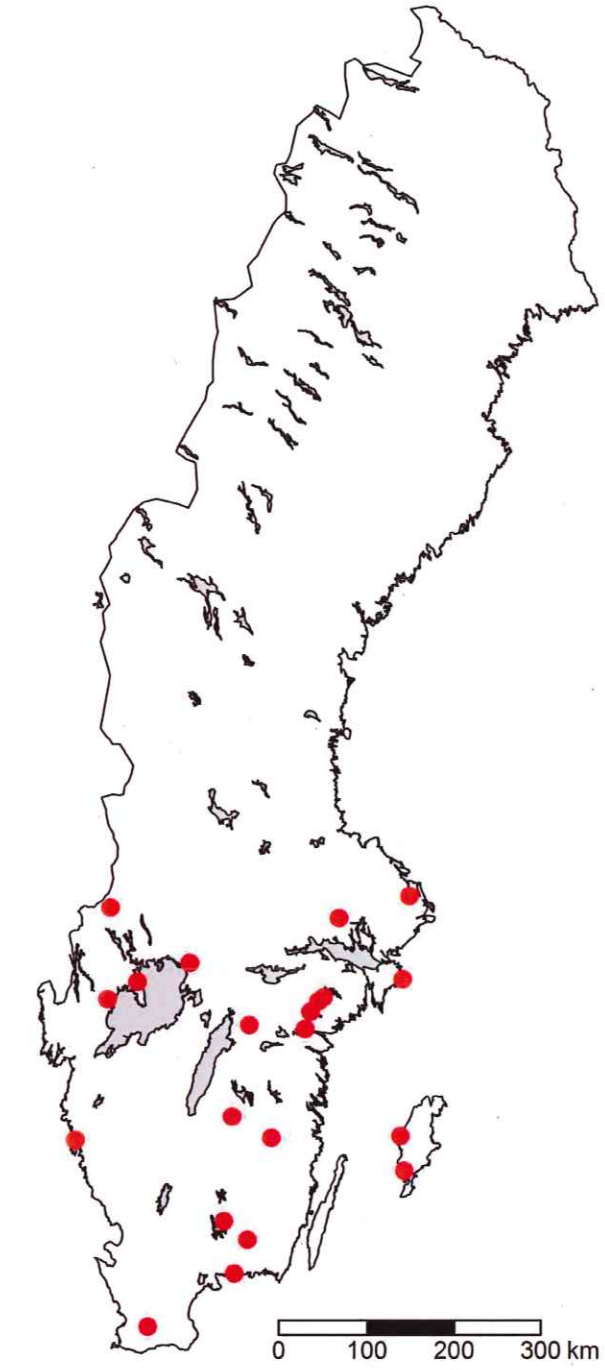


**Figure 1: Map showing Sweden and the 22 study sites in the south of Sweden (red dots).**

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


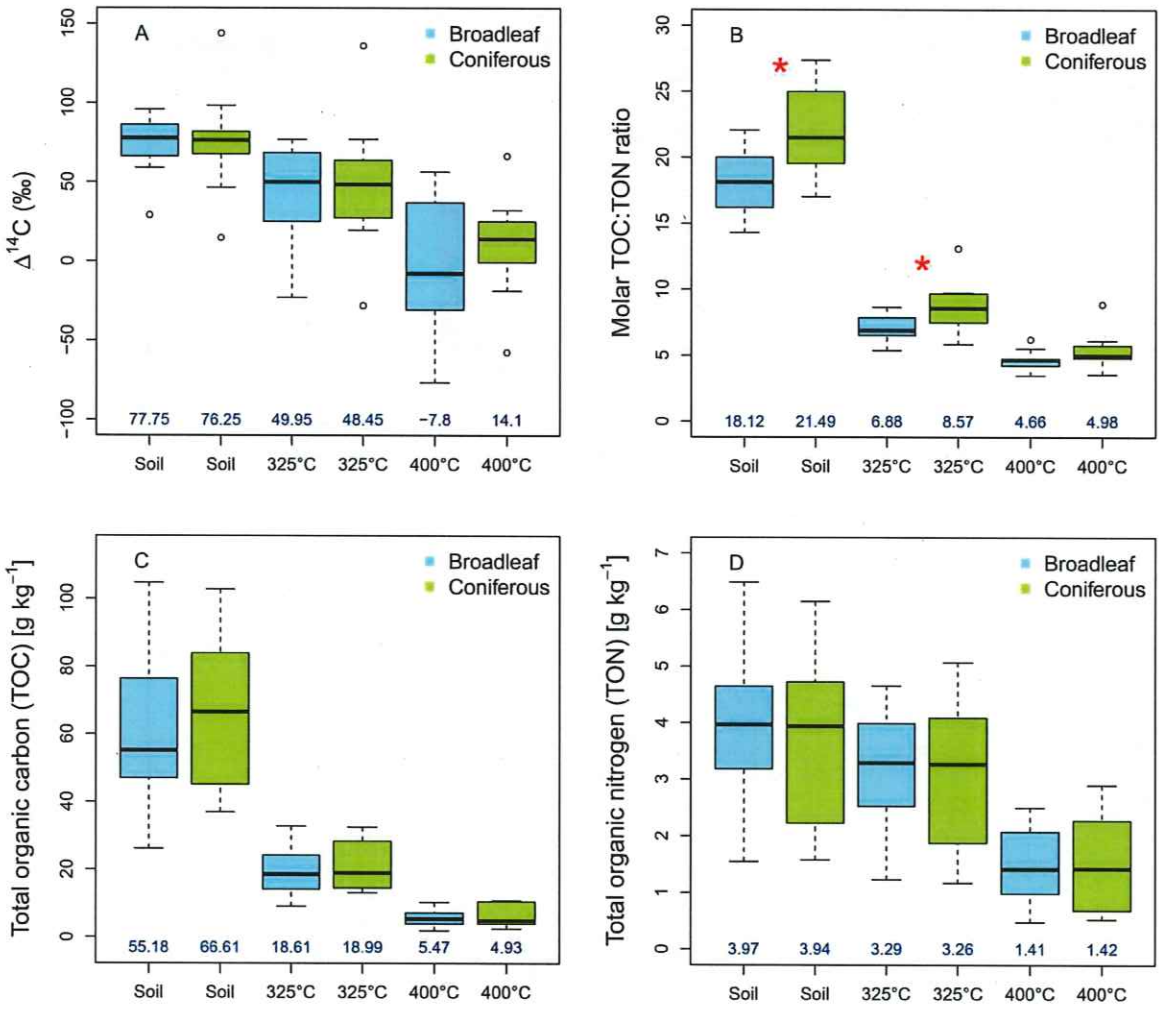


**Figure 2: Properties of soils and thermostable soil organic matter fractions of broadleaf and coniferous forests.** The $\Delta^{14}C$ (panel A), molar TOC:TON ratio (panel B), total organic carbon (TOC; panel C), and total organic nitrogen (TON; panel D) of soils and fractions after ramped thermal treatment up to 325°C and 400°C. Red asterisks indicate statistically significant differences ($p < 0.05$) between the A horizon of broadleaf and coniferous forests according to the student's t-test. The dark blue numbers depict medians. The 25-% and 75-% percentile define the box and the 10-% and 90-% percentiles define the bars.

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


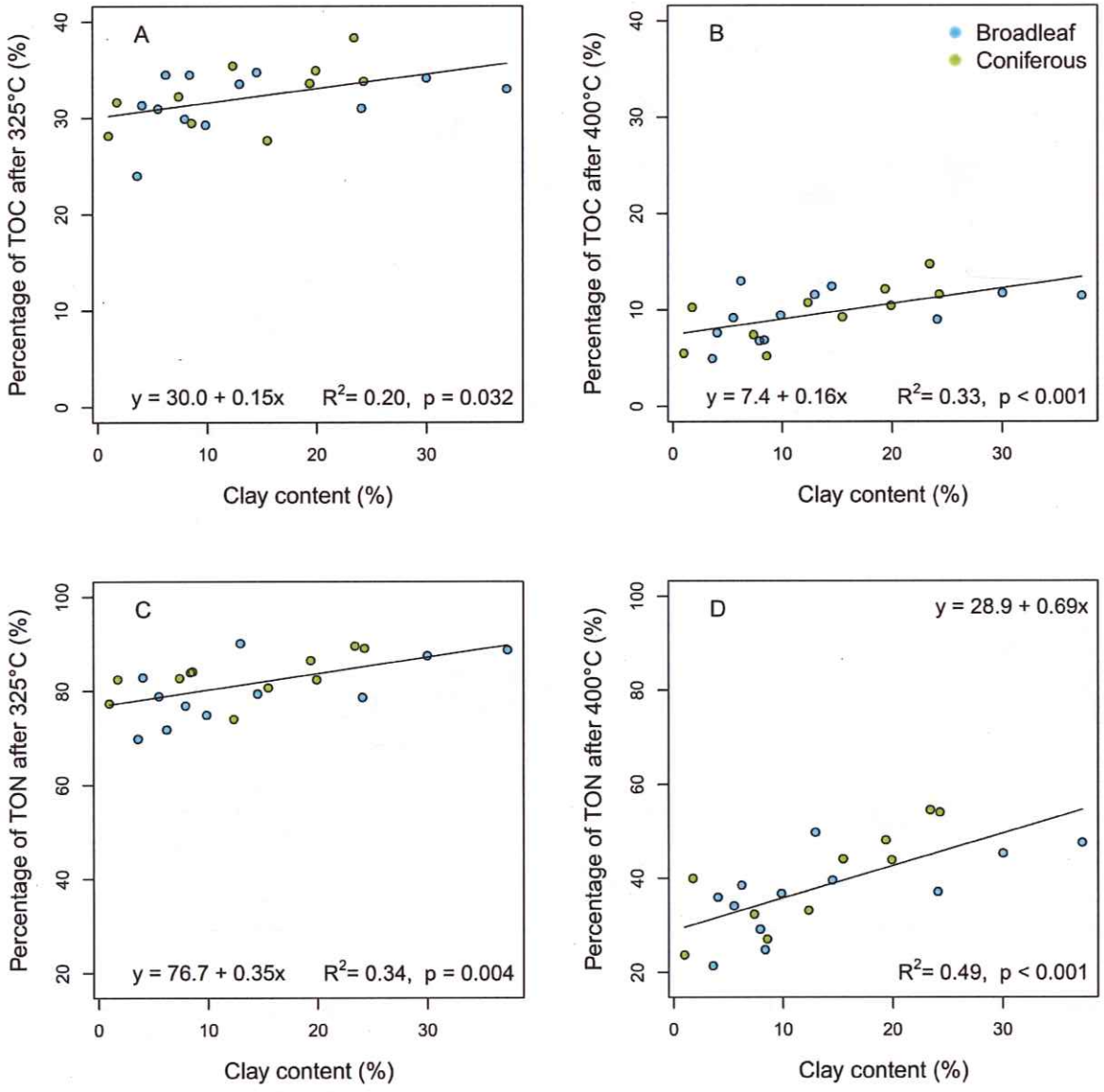


**Figure 3: Relationship between the soil clay content and the percentages of total organic carbon and nitrogen in the thermostable fractions.** The percentage of total organic carbon (TOC, panels A, B) and total organic nitrogen (TON, panels C and D) after ramped thermal treatment up to 325°C (panels A and C) and 400°C (panels B and D) as a function of the soil clay content. The percentages are calculated as percentage of the TOC and TON contents in the untreated soils.

https://doi.org/10.5194/egusphere-2026-3238
Preprint. Discussion started: 12 June 2026


## Data availability

The data of this study is available on Zenodo: https://doi.org/10.5281/zenodo.20540528

## Author contributions

M.S. conceptualized and conducted the research and wrote the manuscript, C.J.O. contributed data and contributed to editing, C.A.S. facilitated radiocarbon measurements and contributed to writing and editing.

## Competing interests

The authors declare no competing interest.

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