# Effects of level and quality of organic matter input on carbon storage and biological activity in soil: Synthesis of a long-term experiment

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[1] The aim of this paper was to synthesize a series of investigations from a long-term field experiment addressing the turnover of organic matter in soil. This paper is based on four organic matter inputs (soil treatments): none (fallow), small amounts of roots+stubble cropped without N fertilizer (no-N), moderate additions of animal manure, and moderate additions of peat. After 42 years, soil carbon stocks declined in the fallow and no-N treated soil but increased in the animal manure and peat-amended soil. Gentle fractionation of soil particles and aggregates revealed that the silt-sized fraction contained most of the soil C and was most responsive to changes in input of organic matter. The clay-sized fraction (<2 μm) acted as a sink for C, and amounts of clay-sized C increased in all treatments, including fallow. The contribution of size fractions to C storage decreased in the following order: silt > clay > fine sand > coarse sand. The highest natural abundance of $^{13}C$ and $^{15}N$ was found in the clay-sized fraction, the fallow being most enriched in $^{13}C$ and the animal manure-treated soil in $^{15}N$, indicating that the organic matter of the clay-sized fraction had been turned over most intensively. The $^{13}C$ inventory showed that the transfer from silt- to clay-sized carbon was most intensive in the soil treated with animal manure and least intensive in the peat-treated soil. Bacterial diversity increased from sand- to clay-sized fractions revealed by 16S rRNA genes. Fungal activity was highest in coarse-sized fractions as indicated by enzyme measurements. The quality and amount of organic matter input had no significant effect on the community structure of soil bacteria. *INDEX TERMS:* 1615 Global Change: Biogeochemical processes (4805); 4806 Oceanography: Biological and Chemical: Carbon cycling; 1055 Geochemistry: Organic geochemistry; *KEYWORDS:* long-term experiment, soil organic carbon, soil organic matter, turnover

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## 1. Introduction

[2] Carbon storage in soil and its response to land-use change affects $CO_2$ concentrations in the atmosphere [*Lal*, 2001; *Smith et al.*, 1997], but significant changes in soil organic carbon contents due to land management practices occur only slowly [*Jenkinson*, 1990; *Campbell et al.*, 2000]. Owing to a high spatial variability of organic carbon in soil, changes cannot be detected with high precision and significance by monitoring only [*Gerzabek et al.*, 2004], and therefore long-term experiments providing reliable data sets of soil organic matter are necessary for detailed studies of soil organic matter turnover [*Johnston*, 1997].

[3] Results from long-term experiments in England (Broadbalk) showed that soils treated with animal manure approached equilibrium concentrations in soil organic matter after about 100 years [*Johnston et al.*, 1989]. Thirteen other European long-term experiments revealed that organic carbon in unfertilized soils range from 0.3% to 1.7% after many decades being roughly proportional to clay content [*Körschens et al.*, 1998]. From a U.S. long-term plot (Sanborn Field), estimates of turnover times for C sources added to soil were derived by measuring natural abundances of stable C and N isotopes [*Wagner*, 1991]. The Ultuna long-term experiment is an agricultural field study in which the influence of different organic matter input over 42 years was examined with respect to the following questions. How do different agricultural practices influence carbon storage in soil? Where is organic matter spatially segregated in soil? How are microorganisms distributed in

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soil? Is the microbial diversity controlled by the quality of organic matter input?

[4] In this paper, results from recent investigations were combined with the information provided by previous publications from the experiment including changes in soil C and N contents over time [*Kirchmann et al.*, 1994; *Gerzabek et al.*, 1997, 1999], size of the soil microbial biomass [*Witter et al.*, 1993], changes of soil physical properties [*Gerzabek et al.*, 1995; *Kirchmann and Gerzabek*, 1999] and responses of soil organic matter [*Gerzabek et al.*, 2001a, 2001b]. A gentle, physical soil fractionation procedure was applied for further investigations of soil size fractions when measuring bacterial diversity using 16S rRNA-based methods, enzyme activities, and natural abundances of $^{13}C$ and $^{15}N$ isotopes. The following hypotheses were tested: (1) Size fractions have different microbial diversity, (2) the silt-sized fraction contributes significantly to the overall carbon storage, and (3) organic matter associated with clay-sized fractions has been turned over to a greater extent than coarser size fractions.

## 2. Materials and Methods

### 2.1. Ultuna Site

[5] The field experiment is in central Sweden near Uppsala (60°N, 17°E; elevation: 14 m above sea level), on a Eutric Cambisol (FAO) with 37% clay and 41% silt. The parent material consists of post-glacial clay dominated by illite. The mean annual temperature is 5.5°C, and the mean annual precipitation is 660 mm. A complete documentation of the experiment and compilation of data are given by *Kirchmann et al.* [1994]. In 1956, the soil (0–20 cm depth) had 15 g kg$^{-1}$ of organic carbon, ($C_{org}$) 1.7 g kg$^{-1}$ of total nitrogen ($N_{tot}$), and a pH of 6.6 ($H_2O$). The area was used as arable land based on a ley-rotation before starting the experiment, animal manure being applied as fertilizer. The experimental design consists of 14 treatments, laid out with four replicates in a randomized block design, the only difference between plots being the type of amendment. Wooden frames separate the individual plots (2 m × 2 m). In the present study we used four of the treatments to represent a wide range of levels and quality of organic matter input. A fallow treatment (continuous bare fallow), which received no C and N input since the start of the experiment, represents a highly soil organic matter depleted soil. A no-N treatment (plots did not receive nitrogen fertilizers) represents a minimum C input through remaining roots and stubble after harvest. In the third treatment, animal manure was applied using well-decomposed cattle manure without additional N fertilizer. These three treatments do not represent high or low input forms of agriculture but are characteristic for agricultural practices worldwide. The fourth treatment was peat, a nonagricultural litter input with a high C/N ratio originating from sphagnum moss. Peat was chosen to introduce a highly deviating C source for scientific examination, representing a historical land-use practice in northern Europe. This treatment did not receive any N fertilizer, either. The application of the organic amendments, animal manure and peat, was based on equal amounts of ash-free organic matter amounting to 2000 kg C ha$^{-1}$ yr $^{-1}$ on average. The organic materials were added by hand in the fall of 1956, 1960, and 1963, and thereafter every second year. Tillage was done by hand to a depth of 20 cm. All plots received annually in spring a dressing of 20 kg P ha$^{-1}$ in the form of superphosphate and 35–38 kg K ha$^{-1}$ in the form of potassium chloride but no inorganic fertilizer nitrogen. Crop rotation consisted of 70% cereals, mainly barley (*Hordeum vulgare* L.) with some oats (*Avena sativa* L.) and spring wheat (*Triticum aestivum* L.), 25% oil seed rape (*Brassica napus* L.) and mustard (*Sinapis alba* L.), and 5% Swedish turnip (*Beta vulgaris* L. var. *crassa*) grown alternately in an irregular order. At harvest, the aboveground portion of the crop was completely removed. Topsoil samples (0–20 cm) were taken in 1956, 1967, and 1974, and thereafter every second year. The composition of animal manure and peat was analyzed prior to soil addition (Table 1).

### 2.2. Fractionation Procedure

[6] Samples from 1998 and archived samples from 1956 were size fractionated based on the method described in detail and tested by *Stemmer et al.* [1998]. To minimize destruction of labile particulate organic matter, the soil-water suspension was dispersed using low-energy sonication (0.2 kJ g$^{-1}$ output energy) followed by successive wet sieving to yield the size fractions >200 μm (coarse sand-sized) and 200–63 μm (fine sand-sized). Thereafter the suspension was centrifuged with an accelerative force of 150 g and 3900 g, respectively, yielding the size fractions of 63–2 μm and <2 μm without addition of a flocculant. Thus fractionation, which was replicated, resulted in a mixture of particles and aggregates and not isolated particles only, which is referred to as size separates in this paper. The fractions were freeze-dried and weighed prior to analysis. The main advantage of this gentle fractionation procedure is that stable microaggregates are preserved and that enzymatic parameters can be measured in the size separates [*Stemmer et al.*, 1999].

### 2.3. Soil Sample Analysis

[7] Organic C and total N in bulk soil samples and size separates were determined by dry combustion in an elemental analyzer (Carlo Erba NA 1500, Milano, Italy). As carbonate C was absent in the soil (pH 5.7–6.7), no acid pretreatment was included. Isotopic abundances were measured by means of an isotope ratio mass spectrometer (Finnigan MAT 251, Bremen, Germany) coupled to the elemental analyzer. The $^{13}C$ and $^{15}N$ results are expressed in the relative δ per mil scale according to the equation $\delta^{13}C$ ($^{15}N$)‰ = $(R_{sample}/R_{standard} - 1) \times 10^3$, where $R = {}^{13}C/{}^{12}C$ ($^{15}N/^{14}N$), and are related to the Pee Dee Belemnite (carbon) or air (nitrogen).

[8] The activity of the enzymes xylanase, urease, and invertase in size separates were estimated by incubation methods using xylane, urea, and sucrose as substrates as described by *Schinner et al.* [1996]. High relative enzyme activities reflect the presence of microorganisms that decompose specific substrates. For the isolation of DNA from soil particle separates, the protocol described by *van Elsas and Smalla* [1995] was used. After purification, total genomic DNA was used as a template to amplify a fragment

**Table 1.** Bulk Soil Properties of the Ultuna Long-Term Experiment in 1956 and 1998 on Four Replicate Plots and Mean Composition of the Organic Amendments[a]

| Soil Treatment | pH ($H_2O$) | $C_{org}$, g kg$^{-1}$ | $C_{mic}$,[b] mg kg$^{-1}$ | $N_{total}$, g kg$^{-1}$ | δ $^{13}$C, ‰ | δ $^{15}$N, ‰ | Bulk Density,[c] kg dm$^{-3}$ | SAS,[d] % | Porosity,[c] Vol % |
|---|---|---|---|---|---|---|---|---|---|
| Initial soil 1956 | 6.5[e] (0.0) | 15.0 (0.3)[e] b | - | 1.70 (0.02)[e] c | −26.3[e] (0.01) | 7.9[e] (0.2) | 1.44[e] | - | 44.6[e] |
| Fallow 1998[f] | 6.2 (0.2) a | 10.8 (0.7) a | 138 (15) | 1.15 (0.08) a | −25.8 (0.1) a | 8.7 (0.2) a | 1.28 (0.00) a | 5.2 (2.3) | 52.0 (0.3) a |
| No-N 1998[g] | 6.5 (0.1) ab | 11.7 (0.6) a | 196 (12) | 1.29 (0.05) b | −26.3 (0.1) b | 8.5 (0.4) a | 1.25 (0.03) ab | 12.8 (0.6) | 53.2 (2.0) ab |
| Animal manure 1998[h] | 6.7 (0.1) b | 22.5 (2.7) c | 422 (38) | 2.18 (0.11) d | −26.9 (0.2) c | 8.7 (0.3) a | 1.16 (0.01) b | 39.2 (3.3) | 55.8 (1.2) b |
| Peat 1998[i] | 5.7 (0.1) c | 32.0 (3.5) d | 290 (20) | 1.75 (0.16) c | −26.3 (1.0) b | 5.4 (0.2) b | 1.03 (0.03) c | 33.3 (3.1) | 59.9 (2.8) c |
| Amendments | | | | | | | | | |
| Animal manure | - | 395 (42) | - | 17.6 (3.0) | −27.5 (0.8) | 7.6 (1.4) | - | - | - |
| Peat | - | 459 (22) | - | 7.7 (1.4) | −25.6 (0.4) | −1.0 (0.9) | - | - | - |

[a]Different letters within columns indicate significance at $P < 0.05$; Numbers in parentheses refer to standard deviation.
[b]Data from *Witter et al.* [1993].
[c]Data from *Kirchmann and Gerzabek* [1999].
[d]SAS means soil aggregate stability; data from *Gerzabek et al.* [1995], only two replicates.
[e]The pH, $C_{org}$ and $N_{total}$ from *Nilsson* [1980]; bulk density and porosity from *Eriksson* [1980]; isotope determination on archived pooled samples.
[f]Fallow since 1956, no N fertilization.
[g]Cropped without N fertilizer.
[h]Cropped without N fertilizer but addition of solid cattle manure amounting to 2000 kg C ha$^{-1}$ yr$^{-1}$.
[i]Cropped without N fertilizer but addition of peat amounting to 2000 kg C ha$^{-1}$ yr$^{-1}$.

of about 470 bp comprising the V6 to V8 variable regions of the 16S rRNA gene using the primers U968/GC and 1385R. The fluorescently labeled terminal-restriction fragment length polymorphism analysis [*Liu et al.*, 1997] used PCR, in which one of the two primers used was fluorescently labeled at the 5′ end (8f-Fam). The RNA isolation and enzyme measurements could only be done on size fractions containing sufficient organic matter; that is, the coarse-sized sand fraction was excluded.

### 2.4. Calculations

[9] Changes in soil bulk densities, which resulted in different thickness of the topsoil layer over the years, were determined on soil cores sampled in cylinders of 72 mm diameter at 0.1-m intervals [*Blake and Hartge*, 1986]. Carbon stocks in topsoils from the plots were calculated using the mass of inorganic soil particles present in the 0- to 20-cm layer at the start of the experiment as a reference unit. Analysis of variance and correlation analyses were done using the program STATISTICA.

## 3. Results

### 3.1. Changes in Soil Organic Matter

[10] Organic carbon concentrations and the size of the soil microbial biomass had changed significantly after 42 years of different treatments (Table 1). Compared to 1956 (15 g $C_{org}$ kg$^{-1}$ soil), the topsoil of the fallow treatment had lost approximately one third of its original $C_{org}$ contents. The no-N plots showed a less distinct decrease, whereas plots treated with animal manure or peat had significantly higher organic carbon concentrations than at the start of the experiment (Table 1). In peat-treated soils, $C_{org}$ concentrations exceeded 30 g kg soil$^{-1}$. Peat addition also increased the C/N ratio from 8.8 to 18.3, which is significantly higher than that of the other treatments (fallow 8.9; no-N 8.9; animal manure 10.7). Size changes of the soil microbial biomass followed $C_{org}$ concentrations of the bulk soil with the exception for the peat-treated soil having a lower ratio of biomass C to soil $C_{org}$.

[11] Amounts of organic carbon in the topsoil declined by 400 kg C ha$^{-1}$ yr$^{-1}$ in the fallow and 530 kg C ha$^{-1}$ yr$^{-1}$ in the no-N treatment (Figure 1) compared with the initial amount of 42 620 kg ha$^{-1}$ in 1956 [*Kirchmann et al.*, 1994]. In the animal-manure- and peat-treated soils with a C input of 2200–2300 kg ha$^{-1}$ yr$^{-1}$ (sum of amendment C plus root and stubble C), soil carbon stocks increased to 57,300 and 69,500 kg ha$^{-1}$, respectively.

[12] The soil organic matter of bulk soil in 1956 had a δ $^{13}$C value of −26.3‰ and a δ $^{15}$N-value of 7.9‰ determined on archived samples (Table 1). The fallow showed a slight but significant increase ($P < 0.05$) in the δ $^{13}$C value. The no-N and peat treatment showed no significant change, whereas the δ $^{13}$C-value of the animal-manure-treatment soil was significantly lower (Table 1). Concerning $^{15}$N, the peat-treated soil had a significantly lower δ $^{15}$N-value of 5.4‰, which was the result of the peat input having a mean δ $^{15}$N-value of −1.0‰ (Table 1).

### 3.2. Soil Carbon in Particle Size Fractions

[13] Size fractionation (Table 2; Figure 1) reveals that the silt-sized separates contained most organic carbon (mean 11.73 ± 1.97 g C kg$^{-1}$ bulk soil) both at the start of the experimental period (1956) and after 42 years, followed by the clay-sized fractions (mean 5.93 ± 0.44 g C kg$^{-1}$ bulk soil). The sand-sized particles contained little soil organic matter in all treatments (Table 2; Figure 1). The changes in C storage in silt-sized fractions followed the changes in $C_{org}$ concentrations in bulk soils ($r$ = 0.986; $P$ < 0.005), whereas the distribution of C in the clay-sized fraction was not significantly correlated with $C_{org}$ in bulk soils.

[14] The natural abundance of both $^{13}$C (mean −25.8‰) and $^{15}$N (mean 9.3‰) values was significantly higher in clay-sized than in coarser fractions (Table 3). Nitrogen added through peat (δ $^{15}$N −1.0‰) had a significant influence on the $^{15}$N abundance; in all size fractions it was the lowest. With the exception of peat, shifts in $^{13}$C and $^{15}$N abundances were greater between particle size fractions than between soil treatments.

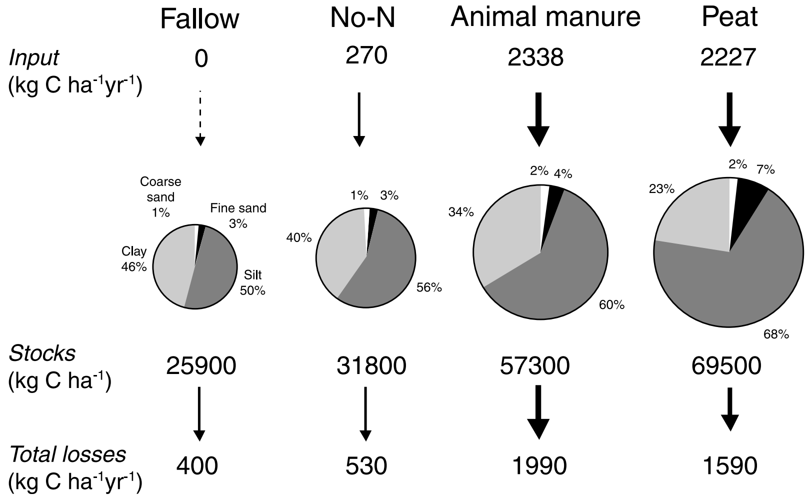


**Figure 1.** Response of carbon pools to four selected treatments in the Ultuna experiment: Carbon input, C partitioning in particle size fractions, C stocks, and total C losses as observed until 1998. The initial amount of soil carbon was 42620 kg $ha^{-1}$at the start of the experiment in 1956. The initial percent C in particle-sized fraction was coarse sand-sized 5%, fine sand-sized 3%, silt-sized 72%, and clay-sized 20%.

[15] The C inventory showed that the clay-sized fraction gained C during the experimental period in all treatments amounting to 68 in the fallow and 100 kg C $ha^{-1}$ $yr^{-1}$ in the no-N treatment (Table 2). On the other hand, the silt-sized fraction of the fallow and no-N treatment lost carbon, on average, 410 and 311 kg C $ha^{-1}$ $yr^{-1}$.The parallel increase of C amounts in the clay-sized fraction of these treatments showed that there was a transfer from the silt-sized to the clay-sized fraction despite an overall decrease of the soil C content.

[16] Additions of animal manure and peat caused significant increases of C amounts in the silt-sized (90 and 407 kg C $ha^{-1}$ $yr^{-1}$) and clay-sized fractions (263 and 174 kg C $ha^{-1}$ $yr^{-1}$), respectively. The $^{13}C$ inventory revealed that the increase of $^{13}C$ amounts in these fractions cannot originate from the sand-sized fractions as the magnitude of loss from the sand-sized fractions would not be sufficient.

[17] Calculation of the C partitioning on a percentage basis (Figure 1) showed that with increasing carbon content in bulk soils, the proportion of C present in silt-sized fractions increased and the proportion of clay-sized C decreased. However, comparing changes in C distribution with those in the initial soil in 1956 showed that clay-sized C fractions gained C in all treatments and the silt-sized fractions lost C. The two treatments with low $C_{org}$ concentrations, fallow and no-N, had especially high proportions of clay-associated organic matter (Figure 1).

[18] Concentrations of C in particle size fractions ranged from 0.15 to about 5% (Figure 2). Sand-sized fractions had the lowest C concentrations (mean 0.58% ±0.43), and silt-sized (mean 2.25% ±1.31) and clay-sized fractions (mean 2.14% ±0.54) had similar concentrations. The fine clay-sized fractions (<0.1 μm) were not considered in the calculation of mean concentration as amounts were very small. Nitrogen concentrations followed the same pattern as C concentrations (data not shown), but more distinctly [*Gerzabek et al.*, 2001b]. As a result, finer-sized fractions with higher C concentrations had lower C/N ratios than coarser fractions. The C/N ratio of organic matter in size separates was significantly negative correlated with C concentrations in particle size fractions ($P < 0.05$) (Figure 2).

### 3.3. Bacterial Diversity and Enzyme Activities in Particle Size Fractions

[19] Soil fractionation showed that sand-sized particles hosted a significantly lower bacterial diversity than finer-size fractions (Figure 3) independent of the soil treatment. Input of animal manure or peat did not seem to increase the bacterial diversity of soils, and fallow did not seem to decrease it. There were no significant differences between treatments.

[20] Concerning enzymes, the no-N and animal manure-treated soil had higher enzyme activities than the fallow and peat-treated soil. The peat-treated soil had significantly lower enzyme activities than the other treatments with the exception for invertase in the sand-sized fraction, probably as a result of a slow organic matter transfer into finer-sized fractions. Urease and xylanase activities were highest in the animal manure-treated soil, probably as a result of the addition of urea and straw through animal manure. The activity of the enzyme xylanase, mainly produced by fungi [*Kandeler et al.*, 2000] decaying hemicellulose, was greatest in the sand-sized fraction and lowest in the clay-sized fraction (Figure 3). The activities of urease and invertase, which are involved in the breakdown of low-molecular compounds, were highest in the silt- and clay-sized fractions. In particular, the clay-sized fraction of the animal-manure-treated soil showed the highest urease activity.

[21] Correlation of the number of terminal restriction fragments with C/N ratios showed that there was a close correlation ($r = 0.441$). This means that bacteria were

**Table 2.** Changes of C in Soil Particle Size Fractions After 42 Years of Different Soil Treatments[a]

| Soil Treatment[b] | Coarse Sand-Sized >200 μm | Fine Sand-Sized 200–63 μm | Silt-Sized 63–2 μm | Clay-Sized <2 μm |
|---|---|---|---|---|
| | *Particles Plus Aggregates, g kg$^{-1}$ Bulk Soil* | | | |
| Initial soil 1956[c] | 36.5 | 153 | 639 | 158 |
| Fallow 1998 | 41.9 a | 170 b | 499 a | 289 b |
| No-N 1998 | 42.5 a | 169 b | 501ab | 288 b |
| Animal manure 1998 | 43.7 a | 157 a | 519 b | 280ab |
| Peat 1998 | 41.0 a | 165ab | 540 c | 255 a |
| | *C Content, g C kg$^{-1}$ Bulk Soil* | | | |
| Initial soil 1956[c] | 0.83 | 0.38 | 11.05 | 3.00 |
| Fallow 1998 | 0.13 (0.02) a | 0.31 (0.10) a | 5.65 (1.06) a | 4.66 (0.29) a |
| No-N 1998 | 0.14 (0.03) a | 0.35 (0.08) a | 6.75 (0.88) a | 4.78 (0.27) a |
| Animal manure 1998 | 0.41 (0.09) b | 0.77 (0.22) b | 12.76 (0.74) b | 7.16 (0.61) b |
| Peat 1998 | 0.60 (0.05) c | 2.07 (0.25) c | 21.77 (6.47) c | 7.13 (0.66) b |
| | *C Inventory, kg C ha$^{-1}$ Topsoil* | | | |
| Initial soil 1956[c] | 2318 | 1061 | 30861 | 8378 |
| Fallow 1998 | 311 (47) a | 746 (240) a | 13613 (2538) a | 11225 (696) a |
| No-N 1998 | 507 (63) a | 922 (209) a | 17784 (2314) a | 12604 (710) a |
| Animal manure 1998 | 1111 (240) b | 2039 (573) b | 34666 (2005) b | 19442 (1662) c |
| Peat 1998 | 1332 (78) b | 4555 (549) c | 47980 (14739) c | 15680 (1450) b |
| | *$^{13}$C Inventory,[d] kg $^{13}$C ha$^{-1}$ Topsoil* | | | |
| Initial soil 1956[c] | 61.4 | 28.8 | 827.0 | 216.1 |
| Fallow 1998 | 8.3 | 19.5 | 358.0 | 285.1 |
| No-N 1998 | 13.9 | 25.3 | 371.2 | 322.6 |
| Animal manure 1998 | 30.5 | 56.8 | 953.3 | 511.3 |
| Peat 1998 | 35.1 | 119.7 | 1233.0 | 402.7 |
| | *Changes in C Inventory, kg C ha$^{-1}$ Topsoil yr$^{-1}$* | | | |
| Fallow 42 years | −48 | − 7 | −410 | + 68 |
| No-N 42 years | −43 | − 3 | −311 | +100 |
| Animal manure 42 years | −29 | +24 | +90 | +263 |
| Peat 42 years | −23 | +83 | +407 | +174 |

[a]Different letters within columns indicate significance at $P < 0.05$.
[b]Soil treatments as in Table 1.
[c]Data from archived pooled samples.
[d]Isotopic data from Table 3.

mainly associated with soil particles with low C/N ratios (Figure 4).

## 4. Discussion

### 4.1. How do the Treatments Influence Soil Organic Matter Turnover?

[22] The fallow soil had the highest enrichment of $^{13}$C in the clay-sized fraction (Table 3). This was due to the fact that native soil organic matter in this treatment had turned over without dilution by fresh, nonhumified organic material that was less enriched in $^{13}$C. The C inventory (Table 2) reveals that silt-sized carbon decreased and clay-sized carbon actually increased over the experimental period. This shows clearly that there is a transfer from silt- to clay-sized carbon during organic matter turnover in soil because there was no interference from organic matter input in this treatment. The clay-sized fraction seems to be the ultimate pool for highly stabilized soil organic matter.

**Table 3.** Isotopic Composition of Soil Particle Size Fractions 1956 and After 42 Years of Different Soil Treatments[a]

| | Coarse Sand-Sized >200 μm, ‰ | | Fine Sand-Sized 200–63 μm, ‰ | | Silt-Sized 63–2 μm, ‰ | | Clay-Sized <2 μm, ‰ | |
|---|---|---|---|---|---|---|---|---|
| Soil Treatment[b] | δ $^{13}$C | δ $^{15}$N | δ $^{13}$C | δ $^{15}$N | δ $^{13}$C | δ $^{15}$N | δ $^{13}$C | δ $^{15}$N |
| Initial soil 1956[c] | −26.5 | 5.1 | −27.2 | 5.0 | −26.8 | 8.5 | −25.8 | 8.9 |
| Fallow 1998 | −26.8 a A | 4.2 a A | −26.2 a AB | 2.4 ab A | −26.3 a AB | 7.6 a B | −25.4 c B | 9.5 a C |
| No-N 1998 | −27.6 c A | 4.8 a A | −27.4 b A | 4.2 bc A | −26.5 b B | 7.3 a B | −25.6 b C | 9.9 ab C |
| Animal manure 1998 | −27.5 c A | 6.6 c A | −27.9 b B | 7.0 c AB | −27.5 c A | 7.5 a B | −26.3 a C | 10.2 b C |
| Peat 1998 | −26.4 b A | 1.4 d A | −26.3 a A | 1.7 a A | −26.5 b A | 4.9 b B | −25.7 b B | 7.8 c C |
| Mean | −27.0 | 4.4 | −27.0 | 4.1 | −26.7 | 7.2 | −25.8 | 9.3 |

[a]Different small letters within columns indicate significance at $P < 0.05$; different capital letters within rows indicate significance at $P < 0.05$ for $^{13}$C and $^{15}$N values, respectively.
[b]Soil treatments as in Table 1.
[c]Data from archived pooled samples.

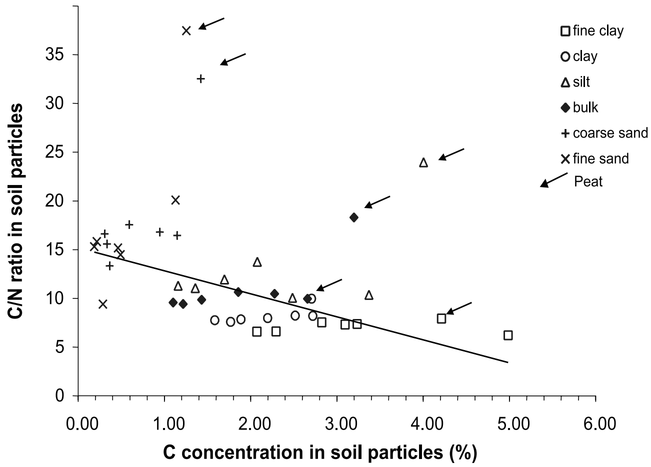


**Figure 2.** Relationship between mean C/N ratios and mean organic carbon concentrations in soil particle size fractions ($y = -2.36x + 15.2$, $r = 0.691$, $P < 0.05$, $n = 37$). Each symbol represents four values. Peat was excluded from the regression analysis.

[23] In the animal-manure-treated soils, the highest $^{15}N$ enrichment was measured in all size separates after 42 years compared to the other treatments. Furthermore, the amount of microbial biomass was largest in this treatment (Table 1), and losses of C were greater than from the other treatments (Figure 1). This indicates that the microbial turnover was most intensive in this treatment.

[24] Owing to the layout of the experiment with equal amounts of organic carbon being added through animal manure and peat, changes in $C_{org}$ concentrations reflect the decomposability of these amendments. Peat was found to be much more recalcitrant than animal manure due to a smaller portion of $C_{org}$ residing in the microbial biomass C (0.9%) (Table 1). Peat also affected the C/N ratio of organic matter in the sand- and silt-sized fractions. The slow microbial turnover of peat is difficult to explain as its C/N ratio or lignin content is not higher than that of the other organic residues [*Kirchmann et al.*, 1994; *Tenney and Waksman*, 1930]. The reason for its recalcitrance is still speculative. A very high abundance of hopane structures has been pointed out (J. Poerschmann, personal communication, 2002).

### 4.2. What Impact do the Long-Term Soil Treatments Have on Carbon Storage?

[25] Our data showed that fallow decreases soil carbon contents to the lowest possible levels. Fallowing without vegetation is a very wasteful strategy that does not enable C sequestration. Fallows are not only a common agricultural practice in nutrient-deficient soils but can also occur after wildfires in nonagricultural systems. Also, land use change from manure-amended to non-fertilized arable soils, which means conversion to extensive farming without animal husbandry and fertilizer input, will decrease the soil carbon stock and increase $CO_2$ emission. This scenario is only realistic if farming for maximum production and financial returns is replaced by standard subsidies to farmers per unit of land. Another important insight was that soil application of peat, a residue formed under waterlogged conditions in wetlands, increases soil carbon contents more than decomposed animal manure. Peat application to soil is only likely in horticultural production today; its use in north European

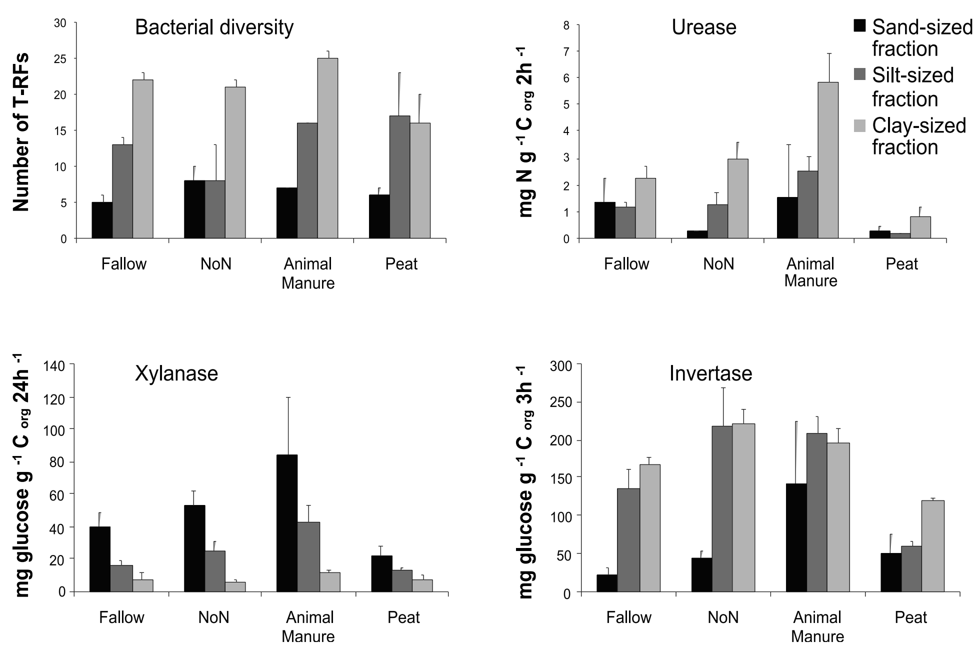


**Figure 3.** Bacterial diversity (measured as terminal restriction fragments, TRF) and microbial activities (measured as the activity of the enzymes urease, xylanase, and invertase) in three soil particle size fractions from the Ultuna experiment (sampling 1998). Low carbon contents in the two coarse-sized fractions meant that analysis was only possible after combining these fractions.

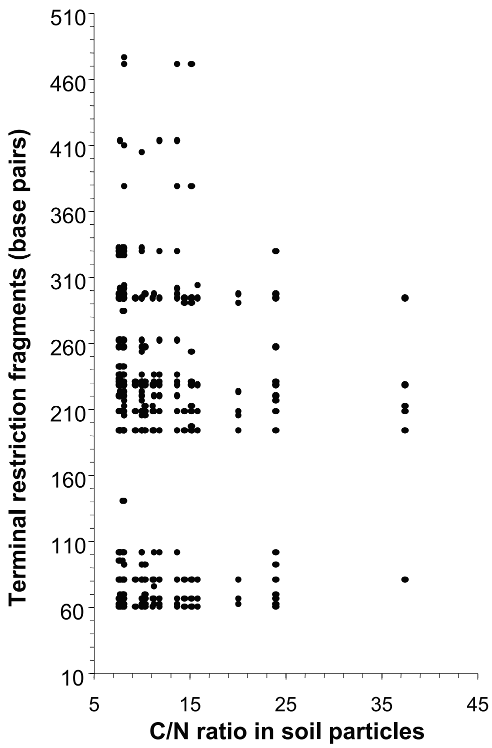


**Figure 4.** Association of bacteria measured as terminal restriction fragments with soil particle fractions that have different C/N ratios ($y = -0.3574x + 20.3$; $r = 0.441$; $P < 0.05$; $n = 22$).

agriculture has stopped. However, although decomposition of peat in the peat-treated soil was low (1590 kg C $ha^{-1}$ $yr^{-1}$), the amount of $CO_2$-C released was 10 times higher than the in situ release of carbon through $CH_4$ plus $CO_2$ from waterlogged mires amounting to less than 100 kg C $ha^{-1}$ $yr^{-1}$ [*Svensson*, 1980].

### 4.3. Is the Silt- or Clay-Sized Fraction the Main Sink for C in Soil?

[26] In our study we applied a low-energy ultrasonic treatment that mainly disrupted macro-aggregates (>63 μm) with little effect on micro-aggregates, which may represent conditions that can occur through normal impacts (e.g., raindrops, plowing). In other fractionation studies, however, soils were treated with high-energy sonication to achieve a complete dispersion [*Christensen*, 1992], and consequently the particle size distribution was similar to the one obtained by chemical pretreatment, and 50–70% of the soil organic matter was present in the clay fraction, while the silt fraction accounted for 20–40% and sand for less than 10% [*Christensen*, 1992; *Amelung et al.*, 1998; *Christensen*, 2000]. Our approach was a prerequisite for the application of more sensitive assays (enzyme, DNA), avoiding redistribution or destruction of biologically active components.

[27] The gentle disruption of soil aggregates resulted in a large silt-sized and a small clay-sized fraction. The silt-sized C fraction was most responsive to organic matter input showing losses of C in the fallow and no-N treatment and gains of C in the animal manure and peat treatment (Table 2). The clay-sized fraction acted as a sink in all treatments. Actually, more carbon was stabilized in the clay-sized than in the silt-sized fraction, with the exception of the peat treatment. Peat particles remain visible in soil for years, and the transformation of peat into soil organic matter is slower than that of other plant and animal residues as indicated by the higher C/N ratio of 18 in the peat-treated soil (Table 1). Peat is highly deviating from agricultural residues due to its recalcitrance to decomposition. One can conclude that the silt-sized fraction is the largest C fraction acting as a buffer and being most sensitive to changes in $C_{org}$ concentrations, whereas the clay-sized fraction stabilizes C independent of whether agricultural residues are applied to soil or not.

### 4.4. Why is the Bacterial Diversity Largest in Finer-Sized Fractions?

[28] Several studies show that the amount of microbial biomass in silt- and clay-sized fractions is larger than in sand-sized fractions [*Jocteur Monrozier et al.*, 1991; *Kanazawa and Filip*, 1986], that finer-sized soil fractions are enriched with sugars derived from microbes, that sand-sized fractions contain sugars of mainly plant origin [*Cheshire et al.*, 1990; *Guggenberger et al.*, 1995], and that lignin in coarse separates is dominated by unmodified plant lignin, whereas lignin components in smaller separates are modified by microorganisms [*Guggenberger et al.*, 1994]. Furthermore, the clay-sized fraction is enriched with teichoic acid, a characteristic biopolymer of bacterial origin [*Rubæk et al.*, 1999]. These results show that small soil particles and aggregates host more microorganisms than coarse-sized particles.

[29] We found that the silt- and clay-sized fraction also favored a greater richness of bacterial species. In ecosystems in general, species richness is controlled by habitat diversity, competition, and predation [*Wardle*, 2002]. In soil, however, the spatial isolation of organisms [*Adu and Oades*, 1978] (less than 0.01% of the soil surface is colonized) may minimize competitive exclusion. Instead, habitat heterogeneity and spatial separation seem to be the main factors promoting richness of microbial diversity in soil. Biomass determinations on soil particle separates showed that more than 50% of the microbial biomass was present in the clay-sized fractions [*van Gestel et al.*, 1996]. This indicates that living conditions for organisms seem to be more favorable on clay mineral surfaces, probably due to the better supply of nutrients and water compared to coarser particles. This also indicates that microbial richness may be greater in small-sized soil fractions. Another example of greater habitat diversity in small-sized soil fractions is provided by *Sessitsch et al.* [2001]: Aerobic and strictly anaerobic bacteria were found in the clay-sized fraction, whereas no strictly anaerobic species were present in sand-sized fractions.

### 4.5. How is Organic Matter Distributed in Soil?

[30] Fractionating soils into particle separates is an arbitrary way to investigate soil characteristics along a size continuum ranging from millimeter to micrometer. We

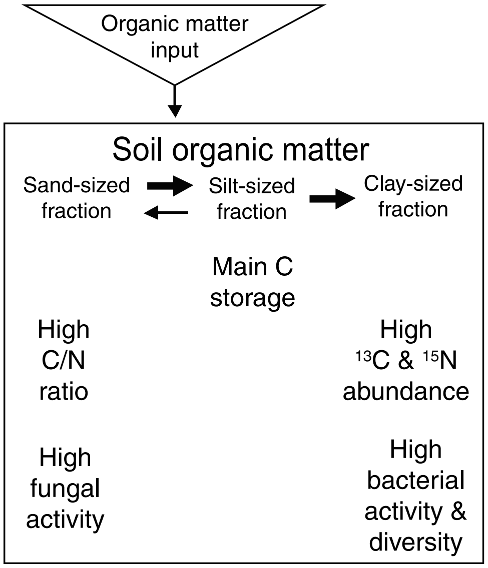


**Figure 5.** Generalized description of organic matter turnover observed in soil particle size fractions.

used the findings derived from fractionation to outline a simple conceptual model (Figure 5) summarizing the turnover of organic matter input to soil.

[31] When new organic matter is added to soil, most of the material is initially located in larger pores formed by sand- and silt-sized particles [*Bergström and Stenström*, 1998]. After the initial decomposition by the microbial community present in the coarse-sized fractions [*Kandeler et al.*, 2000; *Stemmer et al.*, 1998; *van Veen and Kuikman*, 1990], the organic matter is entering smaller pores trapped within aggregates [*Bergström and Stenström*, 1998] increasingly exposed to clay surfaces. This means a transfer from coarser-to finer-sized fractions. A relatively higher natural abundance of $^{13}$C and $^{15}$N in the clay-sized fraction is mainly the consequence of the isotopic discrimination during microbial turnover [*Fernandez and Cadisch*, 2003], but $^{13}$C enrichment may be also accelerated by microbial carboxylation of $CO_2$ from the soil atmosphere [*Cerling et al.*, 1991; *Ehleringer et al.*, 2000]. The gradient of the C/N ratio in the different size fractions reveals the transfer pattern for organic matter from the coarse-sized to the clay-sized fraction. The main habitat for bacterial diversity and activity is the clay-sized fraction, which is a protective habitat through pore size exclusion for predators (protozoa) [*Elliott et al.*, 1980] and a favorable microenvironment due to closeness between nutrients and substrate, whereas fungal activity is highest in coarse-sized fractions.

## 5. Conclusions

[32] Land use change from manure-amended to nonfertilized arable soils, which means conversion to more extensive farming without animal husbandry and fertilizer input, will decrease the soil carbon stock. Fallowing without vegetation is also a wasteful strategy, reducing the soil C storage. A change of the $C_{org}$ concentration in the bulk soil through different land use will also affect amounts of C stored in the clay- and silt-sized fraction. Gentle particle fractionation showed that the silt-sized fraction is the largest carbon storage pool, being most responsive to changes of $C_{org}$ followed by the clay-sized fraction. Furthermore, there is a transfer of silt-sized C to clay-sized C independent of whether organic matter is applied or not. The clay-sized fraction is an ultimate pool for stabilized C with highest natural abundances of $^{13}$C and $^{15}$N. In addition, the C/N ratio decreases with particle size independent of the treatment of soils. The size of soil particles also controls microbial distribution. Coarse-sized fractions host the greatest fungal activity, and bacterial diversity increased with smaller-sized soil particles. The type of land use (different treatments) does not cause significant changes in the bacterial diversity. Soil particles rather than the quality of organic matter used in agriculture affect the turnover of organic matter in soil.

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