*Open Journal of Soil Science*, **2013, 3, 297-313**
Published Online November 2013 (http://www.scirp.org/journal/ojss)
http://dx.doi.org/10.4236/ojss.2013.37035

Scientific Research

# Assessment of Soil C and N Stocks and Fractions across 11 European Soils under Varying Land Uses

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Received August 6th, 2013; revised September 6th, 2013; accepted September 11th, 2013



## ABSTRACT

In this study, we measured the stocks and pool sizes of soil organic carbon (SOC) and total soil nitrogen (TN), and their natural $^{13}C$ and $^{15}N$ abundance across a wide range of temperate European ecosystems. The objectives were to examine any distinct isotope patterns with land use or climate, and how C and N in these different ecosystems are distributed among soil organic matter (SOM) fractions to better predict soil C and N dynamics and longer term persistence. Soils were sampled to 30 cm depth at 11 sites of the Nitro Europe (NEU) network and included four forests, three grasslands and four croplands. Surface soil samples were fractionated using a combined size-density fractionation protocol separating light (LF) from heavy particulate organic matter (hPOM) by density and silt-from-clay-associated SOM by size. Down-profile natural abundance $^{15}N$ patterns pointed towards a closed N cycle in the forest sites, while $^{13}C$ patterns suggested differences in plant water use efficiency across the C3 grassland sites. The forests and grassland sites stored the majority of surface SOC and TN in the LF and hPOM pools. Sustained sequestration of C and N in these rather labile pools will rely on management practices that minimize soil disturbance and increase C input. We also found that the mineral fraction (silt and clay) in the cropland soils stored less C and N per unit of fraction mass compared to the forests and grasslands, which points towards a lower mineral-OM stabilization efficiency of cropland soils. Finally, our study revealed total POM (LF plus hPOM) as a strong predictor of SOC and TN differences, particularly among the non-cropped sites. This study shows that these sites, independent of soil type and climate, store a large fraction of C and N in POM pools that are particularly vulnerable to soil disturbance such as caused by land use change.

**Keywords:** Soil Organic Carbon; Soil Nitrogen; Soil Organic Matter Fractions; Natural $^{15}N$ and $^{13}C$ Abundance

## 1. Introduction

Globally, soils store a tremendous amount of organic carbon (~2300 Pg C) and nitrogen (~140 Pg N) [1], of which the majority is contained in the soil organic matter (SOM). The benefits of SOM for soil fertility are widely recognized due to its contribution to soil structure and aeration, nutrient and water holding capacity. The organic matter content of a soil reflects the balance between plant, animal, microbial and erosional inputs, and losses due to mineralization, leaching and erosion. It is therefore a dynamic property, greatly vulnerable to land use and climate [2] and with important feedbacks to the atmospheric green house gas (GHG) balance and the rate of climate change [3]. The increased demand for accurate soil C and N stock assessments and predictions of C and N changes as a result of land use/cover and climate change has triggered large-scale and long-term measurements of soil C and N stocks and pools globally [4-6]. Such empirical studies provide critical information to quantify the response of soil properties to management and changing climate across regions, ultimately supporting climate change and farm policy, food security, and overall ecosystem health. Monitoring soil C and N stocks is imperative not only for understanding how soils change in response to land use and shifting climate patterns, but also for validating and reducing the uncertainty around estimates of biogeochemical prediction models as Century [7], DayCent [8], Roth C [9] and DNDC [10] commonly used in regional and national GHG inventories. Model outputs typically come with large uncertainties [11,12], which is at least

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partly due to the still limited knowledge about ecosystem processes and properties involved in C and N cycling [13]. Consequently, direct measurements by repeated soil inventories are urgently needed to improve our understanding of C and N dynamics and further constrain model estimates [6,12].

Whole soil C and N stock changes are difficult to detect in the short term as the bulk of SOM is stabilized and has turnover times measured in hundreds to thousands of years [14]. This would require long term measurements to capture modest changes in a robust manner [15]. Soil organic matter fractionations can be helpful in studying long-term C and N dynamics, as they can reveal early indications of changes in SOC and TN stocks [16-19] hence predict how ecosystems will respond to a particular change. A number of physical fractionation methods have been successful in terms of isolating more active dissolved (DOM) and particulate (POM) organic matter pools that are likely to participate in short term soil processes (e.g. nutrient cycling) and respond more rapidly to land use or management changes, from more passive mineral-associated SOM pools that are relatively recalcitrant and more resistant to disturbance [20-22]. Structural analyses have revealed unique molecular characteristics of these SOM fractions based on their degree of decomposition, the dominance of different chemical classes, and the relative contributions from plant and microbially-derived C linking their physical characteristics (*i.e.* how they are operationally defined) to their ecological function [23]. Particulate organic matter fractions, obtained either by particle size (often >53 μm) or density (usually 1.3 - 1.8 g $cm^{-3}$) separation, are composed primarily of identifiable and young plant litter in various stages of decomposition that is chemically similar to its source material, whereas SOM associated with silt and clay minerals (usually < 53 μm), occluded within aggregates, or associated in higher density organo-mineral complexes, is generally further decomposed and more microbially altered [24,25], containing a greater proportion of microbial products and more resistant C as compared with larger or lighter fractions [26]. Assessing how the bulk of SOM in different ecosystems is distributed among these ecologically meaningful fractions can provide insights into SOM behavior upon management or land use change. This will ultimately help decision-making on best management practices to maintain or increase soil C and N. For example, Stewart *et al.* [21] stressed the importance of conservation management (reduced disturbance and increased C input) in the Virginia Coastal Plains to avoid rapid losses of SOC, as most of the change in SOC upon changes in management was observed in the active POM pools.

Soil $^{13}C$ and $^{15}N$ natural abundance measurements are another useful tool forinferring changes in C and Ndynamics and reducing uncertainty in estimates of soil C storage. The natural abundance of $^{13}C$ and $^{15}N$ in SOM has been shown to be an integrative measure of the ecosystem factors and processes that produced it, and its measurement has yielded insight into the dominant processes guiding the biological and physical dynamics in a soil system [e.g., 27]. Soil $^{13}C$ values are closely related to vegetation cover (C3 versus C4 plants) and moisture availability. They can inform about historic vegetation or climatic shifts, and can be used to measure SOC turnover [28]. Mineralization and processes associated with SOM formation induce additional variations in the soil$^{13}C$ abundance, contributing to the often observed $^{13}C$ enrichment with soil depth [29,30]. Soil $^{15}N$ values reflect soil N sources and their fractionation during N transformation [31,32]. Generally, closed N cycles (*i.e.* limited N losses) are expressed by $^{15}N$ patterns with lower $^{15}N$ at the soil surface and relatively higher $^{15}N$ with depth, as has been found in temperate forests not subject to significant N inputs, whereas $^{15}N$ enriched soils suggest open N cycles with greater N losses via fractionating pathways such as nitrification and denitrification [31,32]. Nitrogen fertilizer or atmospheric deposition inputs may complicate the interpretation of natural abundance $^{15}N$ gradients in soil [33].

The aim of our investigation was to assess how C and N in different ecosystems are distributed among SOM fractions to predict longer term persistence and SOM behavior when subject to disturbance, such as changes in climate, management or land use. To do this we 1) assessed SOC and TN stocks as well as $^{13}C$ and $^{15}N$ natural abundance of 11 soil profiles (0 - 0 cm) across Europe, and 2) evaluated the distribution and variability of SOC and TN among SOM fractions in these temperate soils of varying land uses. Land use affects SOC and TN stocks, their distribution among SOM fractions and their $^{13}C$ and $^{15}N$ values across the soil profile. Our hypothesis was that forests and grasslands would store more C and N in the more labile POM fractions compared to croplands. We also expected a more uniform $^{13}C$ and $^{15}N$ pattern across the soil profile of the cropland sites due to tillage, and for the forest sites, a $^{15}N$ pattern indicative of a closed N cycle, with depleted $^{15}N$ values in the surface soils as compared to $^{15}N$ values at deeper depths.

## 2. Materials and Methods

### 2.1. Site Description

Soil cores were collected from 11 sites across Europe during spring and summer of 2007 representing a variety of climates, land-use and soil properties. The sites cover different climatological zones (**Figure 1**) from Finland in the north to Italy in the south, and from the UK in the west to Hungary in the east [34,35] and are part of the Nitro Europe Level-3 "Super Sites" network. Addition-

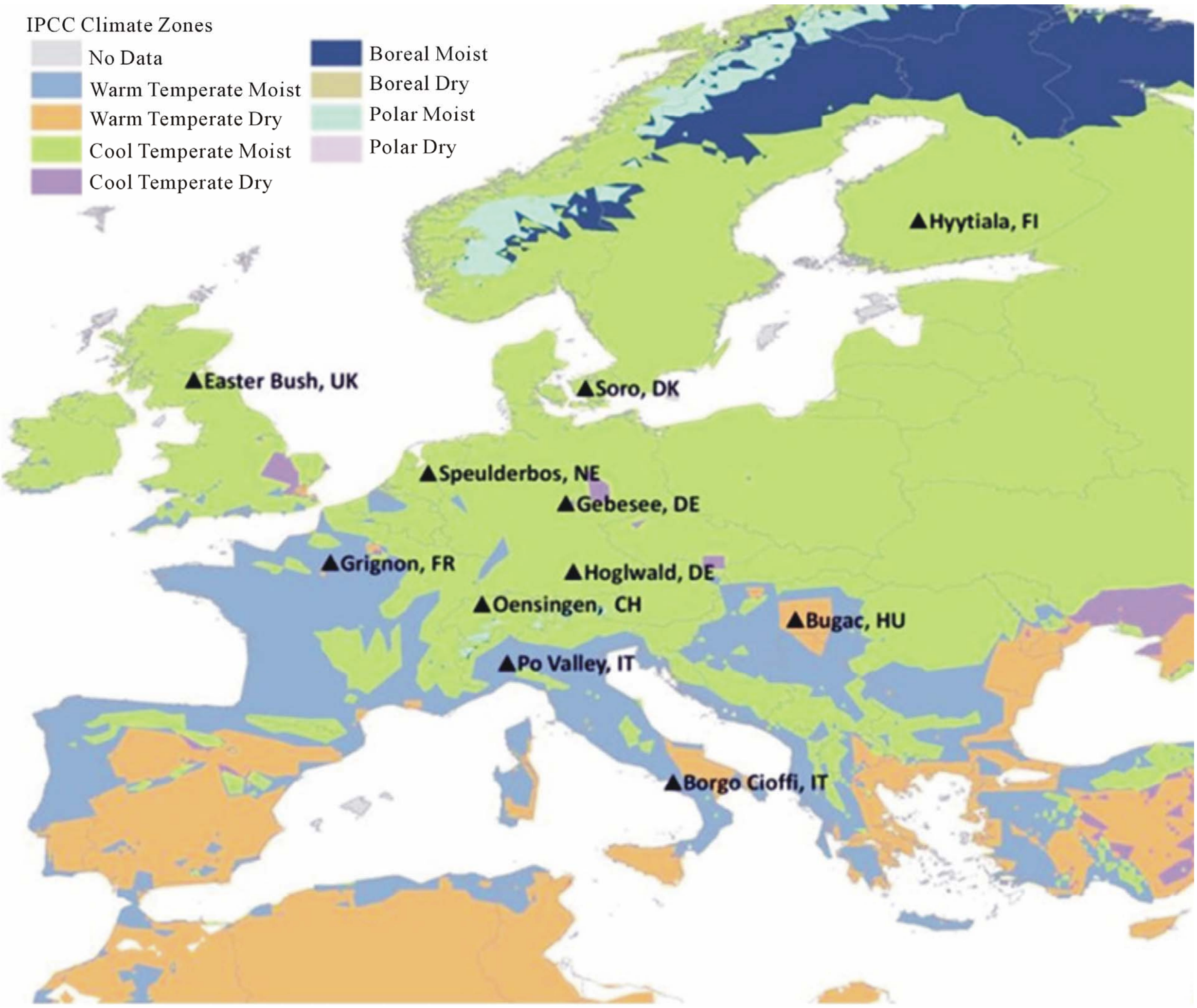



**Figure 1. Map with location of sampled sites and IPCC climate zones.**

ally, the sites also differ in terms of vegetation, soil properties (e.g. bulk density, texture, pH) and land use, including four forests, three grass lands and four croplands. All croplands and grasslands, except for the grassland in Bugac, are fertilized with different amounts of fertilizer. Grasslands are either grazed or mowed. Forest sites differ in tree species of coniferous or deciduous type. The main site characteristics are summarized in **Table 1**. Additional information can be found in Schaufler *et al.* [36] and Skiba *et al.* [35].

## 2.2. Soil Sampling and Preparation

To allow for data comparison and integration with other soil and gas measurements at the NEU Level-3 "Super Site" network [35], soil samples were collected in proximity to the six replicate flux measurement chambers which were randomly placed at each site (for details, see [37]). Around each chamber, after removing the litter layer (where present), one soil core was taken at each of the four corners of a 16 $m^2$ area to a depth of 30 cm with a 5 cm diameter sample probe. Each of the four soil cores was split into four depths (0 - 5 cm, 5 - 10 cm, 10 - 20 cm and 20 - 30 cm) and composited by depth, resulting in one composite sample for each depth for each replicate area (n = 6) per site. Soil samples of all depths were sieved to 2 mm and oven-dried (105˚C) prior to further analyses. One additional soil core was taken at each sampling location for bulk density measurement. Bulk density was measured for each of the four depths, as the ratio of the dry (105˚C) weight of the soil within that depth divided by the volume, measured from the diameter and height of the core, after correcting for any rocks or coarse fragments. No significant core compaction was observed during sampling.

## 2.3. Bulk Soil Analyses

Oven-dried soil samples were finely ground and analyzed for SOC, TN and isotopic composition ($\delta^{13}C$ [‰] and

**Table 1. Sampling sites with information on land use, vegetation, location, climate and soil characteristics.**

| Site | Site code | Vegetation | Site location | Elevation /m a.s.l. | MAT[a]/˚C | MAP[b] /mm | Soil texture[c] Sand-silt-clay /% | pH | N-fert[d] /kg ha$^{-1}$ y$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Cropland sites | | | | | | | | | |
| Borgo Cioffi, Italy | IT-BCi | *Zea mays/Medicago sativa/Foeniculum vulgare/Lolium perenne* | 40˚31’N 14˚57’E | 15 | 19.0 | 500 | 30-22-48 | 7.2 | 545 |
| Castellaro, Italy | IT-Cas | *Zea mays/Oryza sativa* | 45˚12’N 9˚40’E | 87 | 12.7 | 980 | 28-44-29 | 6.9 | 300 |
| Gebesee, Germany | DE-Geb | *Beta vulgaris L./Solanum tuberosum/Brassica napus* | 51˚06’N 10˚55’E | 158 | 9.7 | 510 | 26-61-13 | 6.2 | 31 |
| Grignon, France | FR-Gri | *Sinapis alba/Zea mays/Triticum spp./ Hordeum vulgare* | 48˚51’N 01˚58’E | 73 | 11.5 | 600 | 30-53-17 | 6.7 | 175 |
| Forest sites | | | | | | | | | |
| Hyytiälä, Finland | FI-Hyy | *Pinus sylvestris* | 61˚51’N 24˚17’E | 181 | 3.9 | 710 | 56-16-18 | 3.6 | - |
| Höglwald, Germany | DE-Hog | *Picea abies* | 48˚30’N 11˚11’E | 530 | 8.5 | 890 | 54-32-12 | 3.1 | - |
| Sorø, Denmark | DK-Sor | *Fagus sylvatica* | 55˚29’N 11˚38’E | 40 | 8.3 | 730 | 48-26-26 | 3.4 | - |
| Speulder Bos, The Netherlands | NL-Spe | *Pseudotsuga menziesii/ Quercus robur* | 52˚22’N 05˚32’E | 27 | 9.4 | 460 | 81-18-1 | 2.9 | - |
| Grassland sites | | | | | | | | | |
| Bugac, Hungary | HU-Bug | *Festuca spp.* | 46˚41’N 19˚36’E | 113 | 10.5 | 500 | 93-3-4 | 6.9 | 16 |
| Easter Bush, UK | UK-EBu | *Lolium perenne* | 55˚52’N 03˚12’W | 271 | 9.6 | 850 | 53-26-20 | 4.8 | 171 |
| Oensingen, Switzerland | CH-Oen | *Lolium perenne, Trifolium repens* | 47˚17’N 7˚44’E | 452 | 9.0 | 1100 | 10-47-43 | 5.7 | 230 |

[a]MAT: mean annual temperature; [b]MAP: mean annual precipitation; [c]Sand: 53-2000 µm; silt: 2-53 µm; clay < 2 µm; [d]N fertilizer inputs from N fertilization (IT-BCi, IT-Cas, DE-Geb, FR-Gri, UK-EBu, CH-Oen) or grazing animals (HU-Bug).

$\delta^{15}$N [‰]) using an elemental analyzer (EA) (Thermo Finnigan: EA 1112) coupled with an isotope ratio mass spectrometer (IRMS) (Thermo Finnigan: Deltaplus). Carbonates were removed prior to EA and IRMS analysis by exposure to HCl vapor for 6 hours [38].

## 2.4. Soil Fractionation

All surface samples (0 - 5 cm) were fractionated using a combined size-density fractionation protocol, adopted from Marzaioli *et al.* [39]. This method separates total light fraction (LF) from heavy particulate organic matter (hPOM) by density separation and silt-from clay-associated SOM by size. In brief, 2 mm sieved soil samples underwent a density separation in sodium polytungstate (SPT; $\rho$ = 1.85 g cm$^{-3}$). This step allows the separation of LF (floats in the SPT) from heavy fraction (settles out in the SPT). The heavy fraction, which is composed of aggregates, sand and POM, was dispersed by glassbeads in de-ionized water, and wet sieved on a 53 µm mesh sieve, to separate the hPOM plus sand fraction (retained on the 53 µm screen) from the silt plus clay fraction. Silt and clay sized fractions were separated by means of wet centrifugation (127 g for 7 min for silt, and 1730 g for 15 min for clay following addition of flocculant, *i.e.* 0.25 M $CaCl_2$-$MgCl_2$). All recovered fractions were analyzed for SOC and TN after carbonate removal [38], as described above.

## 2.5. Statistical Analyses

To test the assumption of normality, we examined QQ plots of the quantiles of studentized residuals versus standard normal quantiles. Soil organic C and TN stocks and concentrations were root-transformed to achieve normality. Statistical tests were performed using transformed data, but non transformed values were used to report average values in tables and figures. The effects of land use, climate (mean annual temperature (MAT) and precipitation (MAP)), soil properties (pH, texture) and their interactions on SOC and TN stocks were analyzed using the ANCOVA model with the proc mixed module in SAS 9.2 (SAS Institute). The effects of land use on fraction C and N content were analyzed using the ANOVA model with the proc mixed module. The variable "site within land use" was considered a random effect in all analyses to resolve non-independencies and to account for the by-site variation. Separation of means was tested with Tukey's honestly significant difference at a significance level of 0.05. Linear relationships between total SOC and TN content and individual C and N fractions, isolated by the different fractionation methods, were tested using ordinary least squares linear regression.

## 3. Results

### 3.1. Bulk Soil Organic Carbon and Total Nitrogen

Soil organic C and TN stocks in the 0 - 30 cm depth ranged between 3.80 and 11.19 $kgm^{-2}$ for SOC and 0.17 and 0.90 $kgm^{-2}$ for TN and differed greatly among sites, even within a land use type (**Table 2**; see also **Tables 3** and **4** for individual depths). The highest SOC and TN stocks were found at the Bugac grassland site and Sorö forest site, respectively, while the lowest SOC and TN stocks were found at the Borgo Cioffi cropland site and Hyytiälä forest site, respectively. Over the entire 30 cm depth, SOC and TN stocks did not significantly differ with land use, climate (MAT, MAP), soil properties (pH, texture) or their interactions.

Significant differences between land use types were observed for SOC and TN concentrations at the different depths (**Figure 2**; **Table 5**). The SOC and TN concentration in the surface 0 - 5 cm depth was significantly lower in cropland sites compared to grassland (SOC: $P = 0.0085$; TN: $P = 0.05$) and forest sites (SOC: $P < 0.0001$). At the deepest depth (20 - 30 cm), TOC and TN concentrations were significantly lower in the forest sites compared to grassland and cropland sites ($P < 0.05$). SOC and TN concentration significantly decreased with depth in the grassland and forest sites ($P < 0.0001$), but not in the cropland sites (**Figure 2**).

### 3.2. Bulk Soil 13C and 15N

Delta $^{13}C$ values ranged between −29.9‰ and −20.6‰, whereas $\delta^{15}N$ values ranged between −2.1‰ and 11.0‰ (**Figure 3**). The highest $\delta^{13}C$ and $\delta^{15}N$ values were found at the Borgo Cioffi cropland site, while the lowest $\delta^{13}C$ and $\delta^{15}N$ values were found at the Hyytiälä and Speulderbos forest sites, respectively. At all sites, bulk soil became enriched in $^{13}C$ up to several parts permil with depth (average increase 2.4‰). Similar results were found for $^{15}N$ in grassland (as high as 3.6‰ increase with depth) and forest sites (as high as 10.4‰ increase with depth), but for cropland sites, the $^{15}N$ signature of the soil did not significantly change with depth (**Figure 3**). Land use significantly affected the $^{15}N$ ($P = 0.0147$) but not the $^{13}C$ signature of the bulk soil (**Table 5**). The land use effect on $^{15}N$ was dependent on depth, with forest soils being more depleted in $^{15}N$ in the 0-5 cm depth (as low as −2.1‰ at Speulderbos) compared to the cropland ($P < 0.0001$) and grassland sites ($P = 0.0079$). At deeper depths, no significant differences among land use types were found in the isotopic composition of the SOC and TN.

### 3.3. Carbon and Nitrogen Content of Soil Organic Matter Fractions

Soil organic C and TN were differently distributed among the isolated SOM fractions at the different sites (**Figure 4**). In the majority of forest and grassland soils, total POM (LF plus hPOM) accounted for most of the SOC and TN. Over 60% of SOC and TN were found in total POM in the forest soils at Hyytiälä, Hogwald, Sorö and Speulderbos, and even over 90% were found in the grassland soils at Bugac and Easter Bush (**Figure 4**). The grassland site at Oensingen, on the other hand, had the majority of SOC and TN (80%) stored in the mineral fractions (silt and clay). Cropland soils stored most of their SOC and TN in the mineral fractions, with the exception of the cropland soil at Grignon, which stored nearly 70% of SOC and TN in its POM fractions (**Figure 4**).

When fraction C and N were expressed on a per fraction mass basis, the silt and clay fractions were significantly less enriched in SOC and TN in all the cropland sites (Borgo Cioffi, Castellaro, Gebesee and Grignon) compared to the grassland and forest sites (**Figure 5**; **Table 5**). At most sites, more SOC and TN were associated with clay compared to silt minerals (**Figure 5**).

### 3.4. Relationship between Soil Organic Matter Fractions and Total Carbon and Nitrogen

In order to evaluate the potential of the isolated SOM fractions for predicting SOC and TN changes, we correlated each individual fraction's C and N content against the SOC and TN calculated combining all fractions. A significant linear relationship was found between SOC (and TN) and total POM (LF plus hPOM) C (and N) when all sites were included as well as within a land use type, with the predictive strength ($R^2$) being much greater for the non-cropped sites ($R^2 > 0.85$) compared to the croplands ($R^2 = 0.46$ for TN; $R^2 = 0.71$ for TC) (**Table 6**). No reasonable relationship was observed when correlating total SOC and TN with any of the other fractions (**Table 6**), suggesting that total POM is the largest con-

**Table 2. Total 0 - 30 cm soil organic carbon (SOC) and nitrogen (TN) stocks (kg m$^{-2}$). Values represent averages of 6 field replicates, with standard deviation in brackets.**

| Land Use | Site | SOC stock/kg m$^{-2}$ | TN stock/kg m$^{-2}$ |
|---|---|---|---|
| Cropland | IT-BCi | 3.80 (0.56) | 0.40 (0.08) |
| | IT-Cas | 7.93 (1.13) | 0.73 (0.11) |
| | DE-Geb | 8.61 (0.33) | 0.78 (0.03) |
| | FR-Gri | 9.29 (0.49) | 0.89 (0.07) |
| Forest | FI-Hyy | 4.12 (0.65) | 0.17 (0.04) |
| | DE-Hog | 4.33 (1.26) | 0.24 (0.10) |
| | DK-Sor | 8.42 (2.05) | 0.90 (0.16) |
| | NL-Spe | 9.28 (1.00) | 0.30 (0.07) |
| Grassland | HU-Bu | 11.19 (0.83) | 0.86 (0.07) |
| | UK-Ebu | 8.77 (1.14) | 0.63 (0.13) |
| | CH-Oen | 7.64 (0.28) | 0.77 (0.03) |

**Table 3. Soil organic carbon (SOC) stocks (kg m$^{-2}$) at 0 - 5 cm, 5 - 10 cm, 10 - 20 cm and 20 - 30 cm depths. Values represent averages of 6 field replicates within a site, with standard deviation in brackets.**

| Land Use | Site | 0 - 5 cm/kg m$^{-2}$ | 5 - 10 cm/kg m$^{-2}$ | 10 - 20 cm/kg m$^{-2}$ | 20 - 30 cm/kg m$^{-2}$ |
|---|---|---|---|---|---|
| Cropland | IT-BCi | 0.54 (0.05) | 0.64 (0.19) | 1.40 (0.17) | 1.24 (0.01) |
| | IT-Cas | 1.16 (0.37) | 1.34 (0.38) | 2.17 (0.36) | 3.26 (0.70) |
| | DE-Geb | 1.28 (0.09) | 1.45 (0.22) | 2.90 (0.27) | 2.98 (0.22) |
| | FR-Gri | 1.67 (0.17) | 1.46 (0.28) | 3.43 (0.18) | 2.79 (0.18) |
| Forest | FI-Hyy | 0.99 (0.44) | 1.00 (0.20) | 1.34 (0.58) | 0.77 (0.31) |
| | DE-Hog | 1.90 (0.81) | 0.80 (0.23) | 0.87 (0.14) | 0.76 (0.23) |
| | DK-Sor | 2.42 (0.45) | 2.19 (0.80) | 2.72 (0.93) | 1.09 (0.42) |
| | NL-Spe | 3.92 (0.55) | 1.70 (0.30) | 2.30 (0.38) | 1.36 (0.19) |
| Grassland | HU-Bu | 2.85 (0.88) | 2.10 (0.28) | 4.98 (0.56) | 2.14 (0.75) |
| | UK-Ebu | 1.92 (0.39) | 1.92 (0.52) | 2.72 (0.33) | 2.21 (0.63) |
| | CH-Oen | 1.54 (0.25) | 1.41 (0.20) | 2.35 (0.31) | 2.33 (0.09) |

**Table 4. Total soil nitrogen (TN) stocks (kg m$^{-2}$) at 0 - 5 cm, 5 - 10 cm, 10 - 20 cm and 20 - 30 cm depths. Values represent averages of 6 field replicates within a site, with standard deviation in brackets.**

| Land Use | Site | 0 - 5 cm/kg m$^{-2}$ | 5 - 10 cm/kg m$^{-2}$ | 10 - 20 cm/kg m$^{-2}$ | 20 - 30 cm/kg m$^{-2}$ |
|---|---|---|---|---|---|
| Cropland | IT-BCi | 0.059 (0.009) | 0.009 (0.067) | 0.067 (0.022) | 0.022 (0.142) |
| | IT-Cas | 0.117 (0.037) | 0.037 (0.130) | 0.130 (0.037) | 0.037 (0.195) |
| | DE-Geb | 0.117 (0.010) | 0.010 (0.126) | 0.126 (0.009) | 0.009 (0.280) |
| | FR-Gri | 0.154 (0.013) | 0.013 (0.132) | 0.132 (0.022) | 0.022 (0.339) |
| Forest | FI-Hyy | 0.031 (0.012) | 0.012 (0.035) | 0.035 (0.007) | 0.007 (0.053) |
| | DE-Hog | 0.096 (0.065) | 0.065 (0.044) | 0.044 (0.018) | 0.018 (0.052) |
| | DK-Sor | 0.352 (0.176) | 0.176 (0.196) | 0.196 (0.095) | 0.095 (0.177) |
| | NL-Spe | 0.154 (0.040) | 0.040 (0.049) | 0.049 (0.012) | 0.012 (0.063) |
| Grassland | HU-Bu | 0.291 (0.172) | 0.172 (0.169) | 0.169 (0.038) | 0.038 (0.290) |
| | UK-Ebu | 0.153 (0.028) | 0.028 (0.134) | 0.134 (0.032) | 0.032 (0.189) |
| | CH-Oen | 0.147 (0.024) | 0.024 (0.148) | 0.148 (0.022) | 0.022 (0.227) |

tributor to surface soil C and N differences across the different sites.

## 4. Discussion

Recent research efforts supported by the European Union (e.g., CarboEurope and NitroEurope) have initiated large scale assessments of the C and N fluxes from soils across Europe. Our investigation contributes to this effort by providing SOC and TN stocks for 11 NitroEurope flux measurement sites across Europe [34], and allows for a more in-depth evaluation of how soil C and N is stabilized in these systems by evaluating the distribution of C

**Table 5. Summary of the results of the analysis of variance on SOC and TN concentrations, SOC and TN distribution among fractions (%), and $^{13}C$ and $^{15}N$.**

| Source of variation | Degrees of freedom | *P* |
|---|---|---|
| SOC concentration | | |
| Land use | 2 | 0.09 |
| Depth | 3 | <0.0001 |
| Land use x depth | 6 | <0.0001 |
| TN concentration | | |
| Land use | 2 | 0.13 |
| Depth | 3 | <0.0001 |
| Land use x depth | 6 | <0.0001 |
| $^{13}C$ | | |
| Land use | 2 | 0.2107 |
| Depth | 3 | <0.0001 |
| Land use x depth | 6 | 0.0314 |
| $^{15}N$ | | |
| Land use | 2 | 0.03 |
| Depth | 3 | <0.0001 |
| Land use x depth | 6 | <0.0001 |
| TOC concentration in silt and clay fractions | | |
| Land use | 2 | 0.0003 |
| Fraction | 1 | <0.0001 |
| Land use x fraction | 2 | <0.0001 |
| TN concentration in silt and clay fractions | | |
| Land use | 2 | 0.003 |
| Fraction | 1 | <0.0001 |
| Land use x fraction | 2 | <0.0001 |

**Table 6. Statistical results for linear regressions between total and individual fraction SOC and N in the 0 - 5 cm soil depth.**

| Fraction-C or -N | $R^2$ | *P* | $R^2$ | *P* |
|---|---|---|---|---|
| | Versus total fraction SOC | | Versus total fraction TN | |
| Total POM | 0.84 | <0.0001 | 0.93 | <0.0001 |
| *Cropland* | 0.71 | <0.0001 | 0.46 | 0.0003 |
| *Forest* | 0.91 | <0.0001 | 0.94 | <0.0001 |
| *Grassland* | 0.85 | <0.0001 | 0.92 | <0.0001 |
| Total mineral (silt + clay) | 0.17 | 0.0006 | 0.01 | ns |
| LF | 0.44 | <0.0001 | 0.26 | 0.0005 |
| hPOM | 0.47 | <0.0001 | 0.02 | ns |
| Silt | 0.16 | 0.0008 | 0.003 | ns |
| Clay | 0.11 | 0.007 | 0.0008 | ns |

and N among SOM fractions. This level of detail can inform on how SOM will behave when ecosystems undergo changes in management or land use, ultimately helping decision-making on how to best preserve or increase soil C and N. Furthermore, our investigation serves the broader objectives of the NitroEurope (NEU) project, by providing a dataset that can be integrated with other soil and gas flux measurements, enabling full C and N budget assessments at the different measurement sites. Such assessments will generate extensive empirical field data from widely different ecosystems across Europe which can be used in the validation of biogeochemical process models such as DNDC, RothC or DayCent.

## 4.1. Total SOC and TN Stocks

In the mineral soil surface (0 - 5 cm), SOC and TN stocks were significantly lower in cropland compared to the other land use systems, as has been found in other studies [e.g.,16,40]. This was a direct result of lower soil C and N concentrations and not just a mere effect of differences in bulk density (**Figure 2**). The loss of surface SOC and TN in cropped soils has been linked to lower C inputs to the soil, the removal of crop residues, reduced vegetation cover, enhanced mineralization of SOM, erosion and deteriorated soil aggregation. Whereas SOC and TN concentrations were rather uniform across the 0 - 30 cm profile in the croplands, a clear stratification with depth was observed in the non-cropped systems. Similar differences in profile distribution of SOC among different land uses (*i.e.* more stratified with depth under conservation management than under conventional cropping) were reported by Franzluebbers [41] using an extensive soil survey dataset in Georgia USA, and suggests that SOC stratification should be viewed as an improvement in soil quality.

For assessing whole-ecosystem responses to management, deeper soil depths need to be included to avoid underestimation of total SOC and TN stocks in intensively managed ecosystems [42]. No significant land use effect on SOC and TN stocks could be discerned when considering the entire 0 - 30 cm depth. Against our expectations, we even found higher SOC stocks in a few cropland sites than in some of the forest and grassland soils. This is in contrast to other inventories reporting distinct differences of SOC stocks between cropland and grassland/forest. For example, the European SOC inventories reviewed by Wiesmeier *et al.* [43] that were restricted to the 0 - 30 cm depth, showed on average 40% and 43% lower SOC stocks for cropland compared with grassland and forest, respectively. The large differences across the sites in our study in terms of soil properties, climate, vegetation and management history could have obscured any effects of land use on total profile SOC and TN stocks. Also, our investigation excluded the litter layer. In forests, the O-horizon can account for a large portion of the total SOC stock. For forests in South-East Germany, Wiesmeier *et al*. [43] found 2.5 kg $m^{-2}$ SOC to be stored in the O horizon, or about 35% of what was stored in the A, B and C horizons combined. In addition, C and N contained in litter fragments > 2 mm were not included

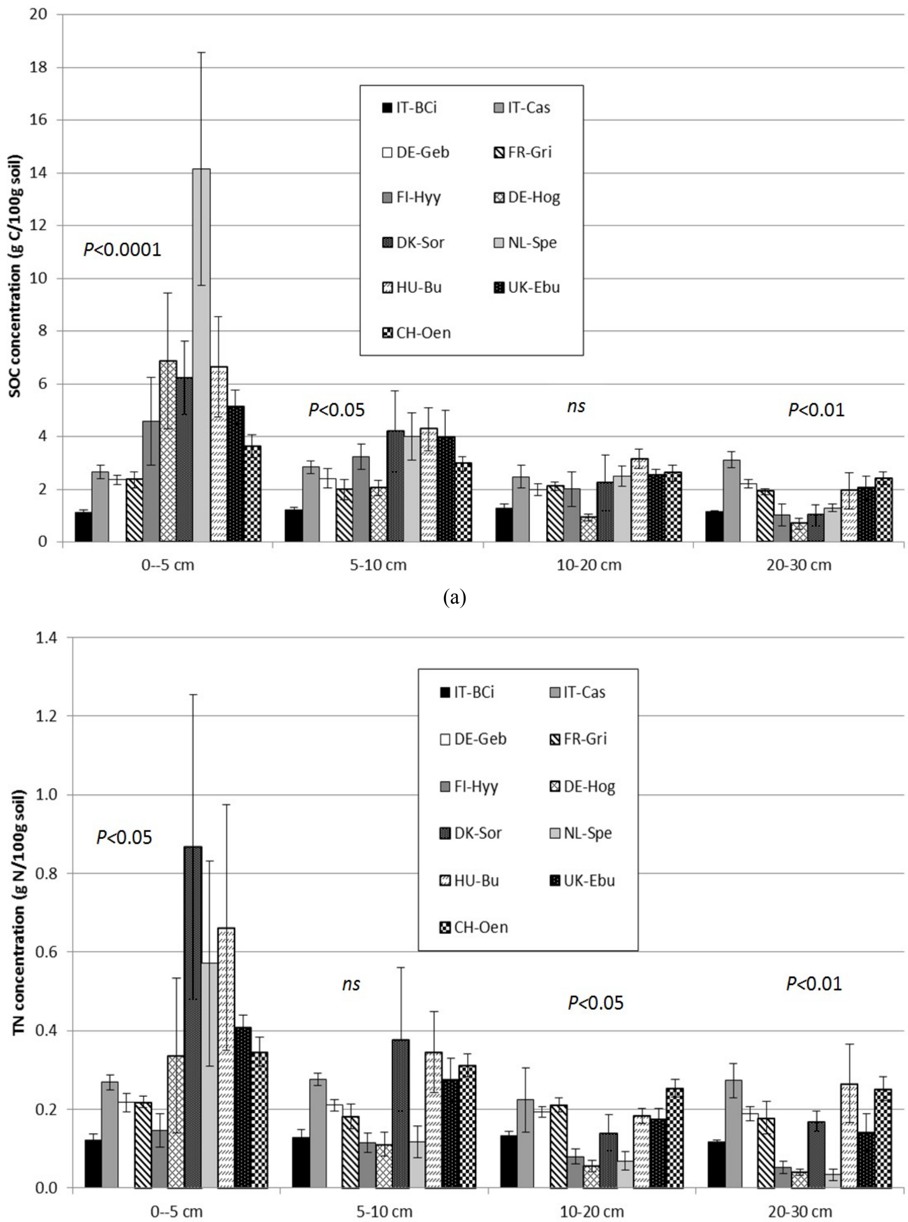



**Figure 2. Soil organic carbon (a) and total nitrogen (b) concentrations (g C or N/100 g soil) in each sampling depth. Bars and error bars represent means and standard deviations of six replicate samples within a site. Sites code is as in Table 1. Statistical results are presented for land use effects per depth.**

in this study, but could contribute to a significant part of the stocks in grassland and forest ecosystems.

Unlike other continental studies [e.g., 2], no clear relationships were found between SOC and TN stocks and any of the climate or soil parameters available for these sites, likely due to the relatively large number of influencing parameters compared to the number of sites included in this study. The highest SOC stocks were found at the Bugac grassland site, which is characterized by a dry-warm continental climate and low-intensity management in terms of grazing and N inputs. These conditions could promote high C inputs while keeping SOM decomposition rates relatively low. The lowest TN stocks were found at the Hyytiälä coniferous forest site, which is characterized by severe N limitation potentially as a result of the low N deposition compared to other forest sites [36,44].

## 4.2. 13C and 15N Abundance

The $^{15}N$ pattern in the forest soils with negative $^{15}N$ in the surface mineral soils and $^{15}N$ enrichment at deeper

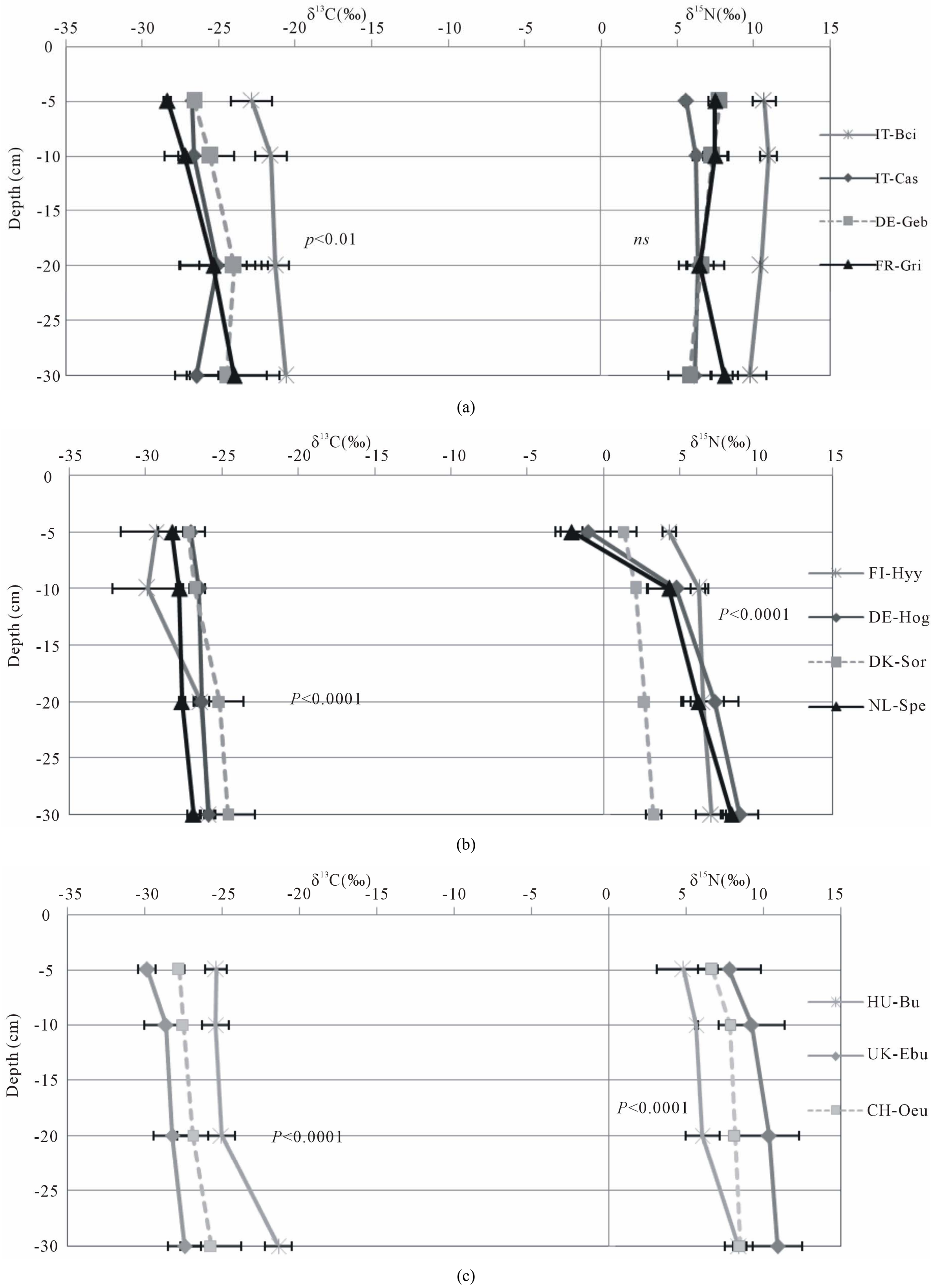
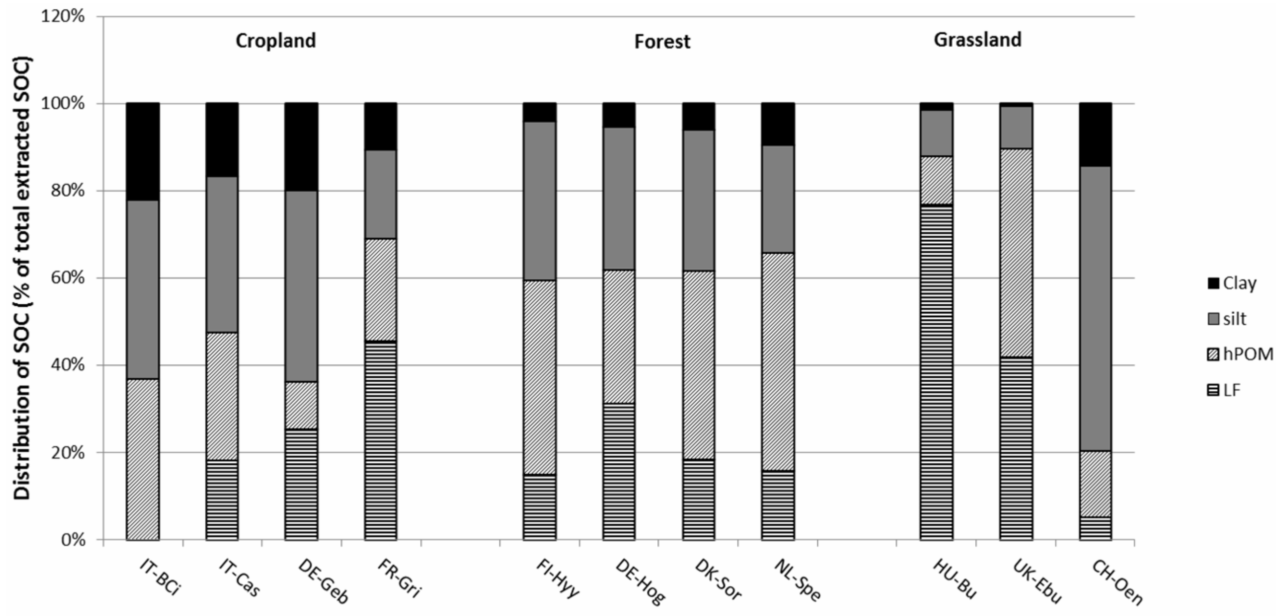
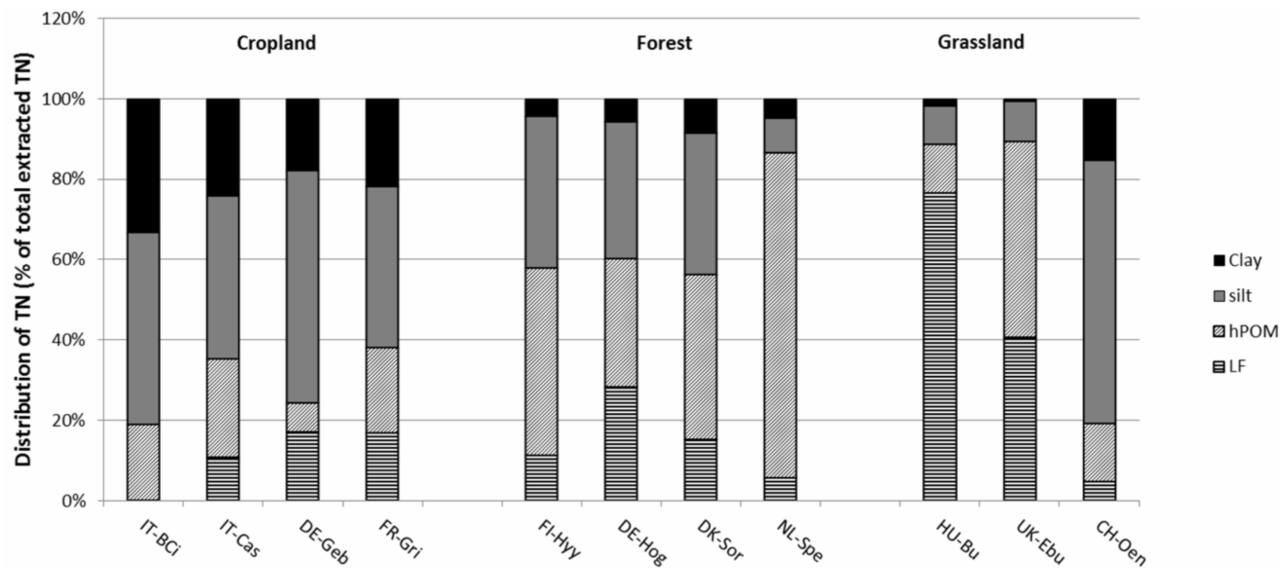



**Figure 3. Delta $^{13}C$ (‰) and delta $^{15}N$ (‰) values across depths for cropland (a), forest (b) and grassland (c) sites. Symbols and error bars represent means and standard deviations of six replicate samples within a site. Sites code is as in Table 1. Statistical results are presented for depth effects per land use.**




(a)

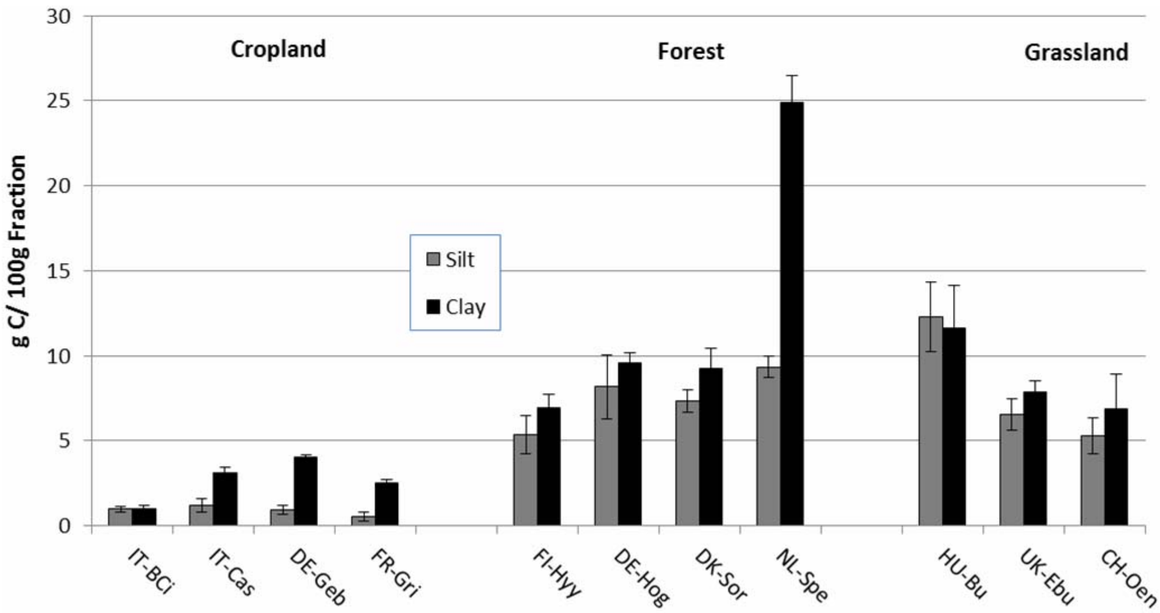



(b)

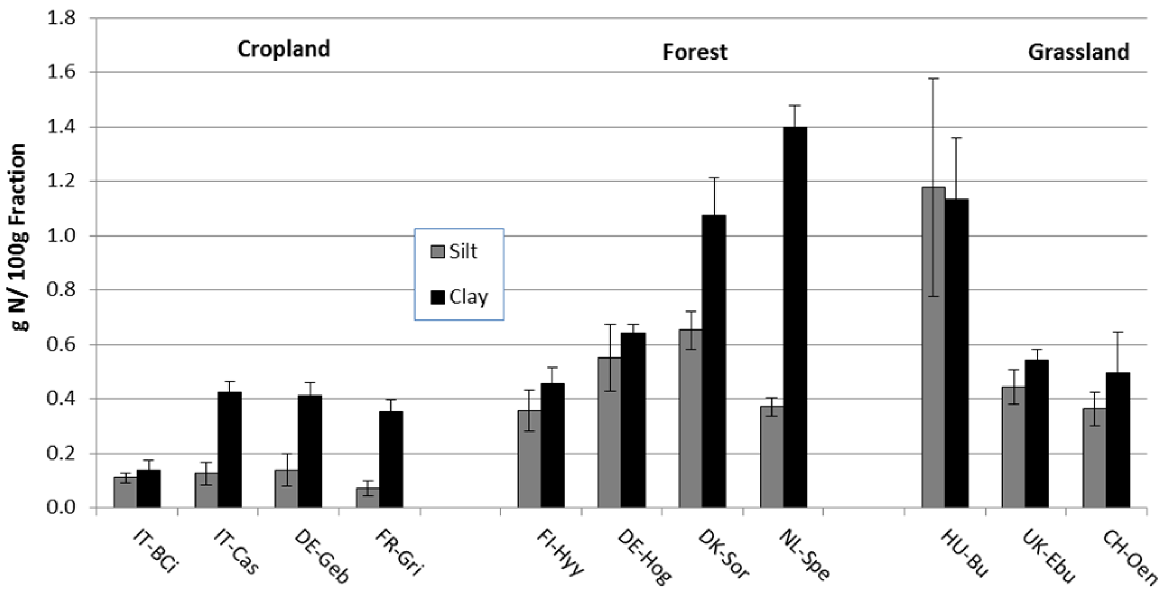
**Figure 4. Proportion of soil organic carbon (a) and total nitrogen (b) (%) in isolated soil organic matter fractions (light fraction, LF; heavy particulate organic matter, hPOM; silt; clay) in 0 - 5 cm depth. Bars represent means of six replicates within a site. Sites code is as in Table 1.**

depths supports our hypothesis of a closed N cycle as has been found in many other temperate forests [31,32]. The higher $^{15}$N values in the surface layers of the grassland and cropland soils suggest greater losses of soil N at these sites through processes which discriminate against $^{15}$N in soils. In systems that receive N inputs from fertilizer or animal grazing, soil $^{15}$N values may also be indicative of the dominant source of soil N. The more enriched $^{15}$N values at Easter Bush compared to the other grassland sites could suggest greater N inputs from animal manure [45] due to the more intense grazing at this site [36]. Similarly, the greater $^{15}$N values at Borgo Cioffi compare to all other cropland sites could be a result of the use of animal waste as an organic fertilizer for the winter fodder crop at this site [46] owing to the preferential volatilization of $^{14}$N ammonia from manure [45].

$^{15}$N enrichment with soil depth, as found in all forest and grassland sites, is a common observation which has been related to different mechanisms, including $^{15}$N isotope discrimination during microbial N transformations, differential preservation of $^{15}$N-enriched SOM components during N decomposition, and more recently to the




(a)




(b)

**Figure 5. Concentration of soil organic carbon (a) and total nitrogen (b) in isolated silt and clay fractions (g C or N/100 g fraction). Bars and error bars represent means and standard deviations of six replicate samples within a site. Sites code is as in Table 1.**

buildup of microbial $^{15}$N-enriched microbial necromass [47]. The lack of $^{15}$N variation within the soil profiles of the cropland soils are probably due to mixing of soil layers by plowing. However, this mixing effect was not apparent for $^{13}$C, which showed a slight enrichment with depth in all sites, independent of land use. The observed variation in $^{13}$C within a depth layer among the cropland sites is likely a result of the different amount of C4 relative to C3 plant C inputs at these sites. The $^{13}$C of the grassland sites, all cultivated with C3 grasses, increased from wetter to drier sites, which could be driven by soil moisture differences and associated differences in plant water use efficiency. Plants grown under water stress become more water-use efficient and exhibit higher $^{13}$C values than plants grown under adequate moisture conditions [48]. The highest $^{13}$C values were found at Bugac, which is characterized by a warm temperate dry climate (**Figure 1**), likely causing higher evapotranspiration and water use efficiency (hence lower $^{13}$C discrimination in C3 plants) than at the cool temperate moist sites at Easter

Bush and Oensingen.

## 4.3. C and N Distribution among SOM Fractions

The soil fractionation data revealed some interesting and clear trends across the different land-use systems, despite the lack of clear trends in the whole-soil, and is a primary reason to fractionate soils [49]. Undisturbed land uses, such as forest and grassland sites (with the exception of Oensingen) stored the majority of SOM in the POM fractions (LF and hPOM), whereas cropland sites (with the exception of Grignon) stored relatively more SOM in the mineral (silt and clay) fractions. Similar results were reported by Franzluebbers & Stuedemann [50] who found lower POM-C as a proportion of TOC in cropland comred to pasture and forest. These observations suggest that less disturbed ecosystems accumulate relatively more C and N in POM fractions, likely as a result of higher C inputs and slower decomposition rates. The distribution of TOC and TN at the grassland site in Oensingen was more similar to that of the croplands with the majority (80%) contained in the mineral fraction. This may be due to the history of intense cropping at this site, with conversion to grass-clover only in 2001, *i.e.* six years prior to soil sampling [51].

Another possible cause for the relatively higher proportion of mineral-associated OM at Oensingen and most cropland sites compared to the other sites could be due to differences in soil texture. Much finer soil textures were found in the cropland sites (between 70% and 74% silt and clay) and Oensingen (90% silt and clay) than at the other sites, hence more mineral surfaces are available in these soils for SOM to accumulate. In fact, a negative relationship between soil texture, as represented by whole-soil silt plus clay content, and the percentage of organic C stored in the POM fraction was observed across all study sites ($P = 0.007$; $r^2 = 0.58$).

Our results are in contrast to other studies, where the mineral-associated OM fraction accounted for most of the total C content independent of land use [e.g., 40]. Relatively high POM proportions have been reported for grassland and forest soils but typically not more than 40% [22,50,52]. This suggests that the European ecosystems included in our study appear to store large amounts of C and N in a rather labile SOM fraction that could potentially be rapidly lost if soils were to be disturbed (e.g., when converted to cropland) [22,50]. The large amounts of POM-C and -N in these systems could also suggest that the mineral (silt and clay) fraction in these systems is C and N saturated [53-55] and cannot store additional C and N inputs. This saturation idea is supported by the higher C and N concentrations in the silt and clay fractions on a per-fraction mass basis in the grassland and forest soils than in the cropland soils (**Figure 5**), suggesting that the non-cropped systems were closer to or at saturation. To further explore this idea, we calculated the theoretical soil C protective capacity of the different sites based on the linear regressions between soil texture and mineral (silt + clay) C content (g kg$^{-1}$ soil) developed by Six *et al.* [54] for different land uses and clay mineralogies. We used the <50 μm regression equations of Six *et al.* [54] for cultivated, grassland and forest to calculate the silt + clay protective capacity (g C kg soil$^{-1}$) from <53 μm silt + clay contents obtained in the soil textural analyses performed on our soils (**Table 6**). These values were then used to derive an estimate of C saturation deficit of the different soils (**Table 6**) based on Stewart *et al.* [55] as follows:

$$C \text{ saturation deficit} = 1 - \frac{\text{Measured silt} + \text{clay } C \text{ content}}{\text{Protective capacity estimate}}$$

with values closer to 1 indicating a lower degree of silt + clay C saturation, and values closer to 0 indicating a higher degree of silt + clay C saturation.

For all forest and grassland sites, with the exception of Easter Bush, the silt + clay C protective capacity estimates were within the 95% confidence intervals of the measured silt + clay C values (**Table 7**), indicating some degree of silt + clay C saturation in these sites. In contrast, the estimated silt + clay C protective capacity of the cropland soils were greater than the measured silt + clay C values (**Table 7**). Correspondingly, greater C saturation deficits were calculated for these soils (0.43 - 0.72) compared to the forest soils (−1.26 - 0.07) and grassland soils (0.01 - 0.30, with the exception of Easter Bush: 0.72). These results support the idea that most forest and grassland ecosystems were closer to mineral C saturation, so that any additional C inputs will accumulate in the more labile POM fractions. Hence, any increase in SOC would be captured in differences in the non-mineral-associated OM pools, which was confirmed by the strong correlation between total SOC and total POM (LF + hPOM) (**Figure 6**), and by the lack of a similar correlation with the mineral-associated SOM fractions (**Table 6**). A linear relationship between POM-C and TOC was also found by Franzluebbers and Stuedeman [50] for pastures in the Southern Piedmont of the USA, whereas the relationship between non-particulate organic C and TOC flattened off, suggesting mineral C saturation beyond which TOC preferentially accumulates in the POC pool. This would also mean that upon any shift in land-use or any soil disturbance, these ecosystems risk losing significant amounts of SOC from enhanced POM decomposition. If these systems can unlimitedly accumulate C and N in POM pools is not clear and the existence of a saturation level for these pools remains to be tested. Yet, there are some indications that the POM pool can become saturated, in particular through aggregate-protected C saturation [54, 56].

**Table 7. Estimates of (1) soil C protective capacity (g C kg$^{-1}$ soil) using relationships between texture (% silt + clay) and mineral (silt + clay) C content (g C kg$^{-1}$ soil) developed by Six *et al.* [54] and (2) C saturation deficit (g C kg$^{-1}$ soil) based on Stewart *et al.* [55], for the 0 - 5 depth of the different soils. Values in between brackets represent 95% confidence interval.**

| Land Use | Site | Estimated C protective capacity | Measured silt + clay C | Estimated C saturation deficit |
|---|---|---|---|---|
| | | /g C kg$^{-1}$ soil | /g C kg$^{-1}$ soil | |
| Cropland | IT-BCi | 21.18 (±5.84) | 6.14 (±0.1.20) | 0.71 (0.60; 0.77) |
| | IT-Cas | 21.78 (±5.96) | 10.13 (±2.02) | 0.54 (0.36; 0.63) |
| | DE-Geb | 19.98 (±5.60) | 11.40 (±2.38) | 0.43 (0.21; 0.55) |
| | FR-Gri | 21.18 (±5.84) | 5.91 (±1.76) | 0.72 (0.61; 0.78) |
| Forest | FI-Hyy | 22.00 (±7.93) | 20.47 (±4.32) | 0.07 (−0.46; 0.32) |
| | DE-Hog | 26.80 (±9.53) | 36.92 (±11.39) | −0.38 (−1.14; −0.02) |
| | DK-Sor | 28.72 (±10.17) | 26.64 (±11.54) | 0.07 (−0.46; 0.32) |
| | NL-Spe | 20.80 (±7.53) | 47.11 (±5.84) | −1.26 (−2.55; −0.66) |
| Grassland | HU-Bu | 18.57 (±5.18) | 13.00 (±8.50) | 0.30 (0.03; 0.45) |
| | UK-Ebu | 31.05 (±7.91) | 8.64 (±2.49) | 0.72 (0.63; 0.78) |
| | CH-Oen | 45.13 (±10.99) | 44.71 (±7.88) | 0.01 (−0.31; 0.20) |

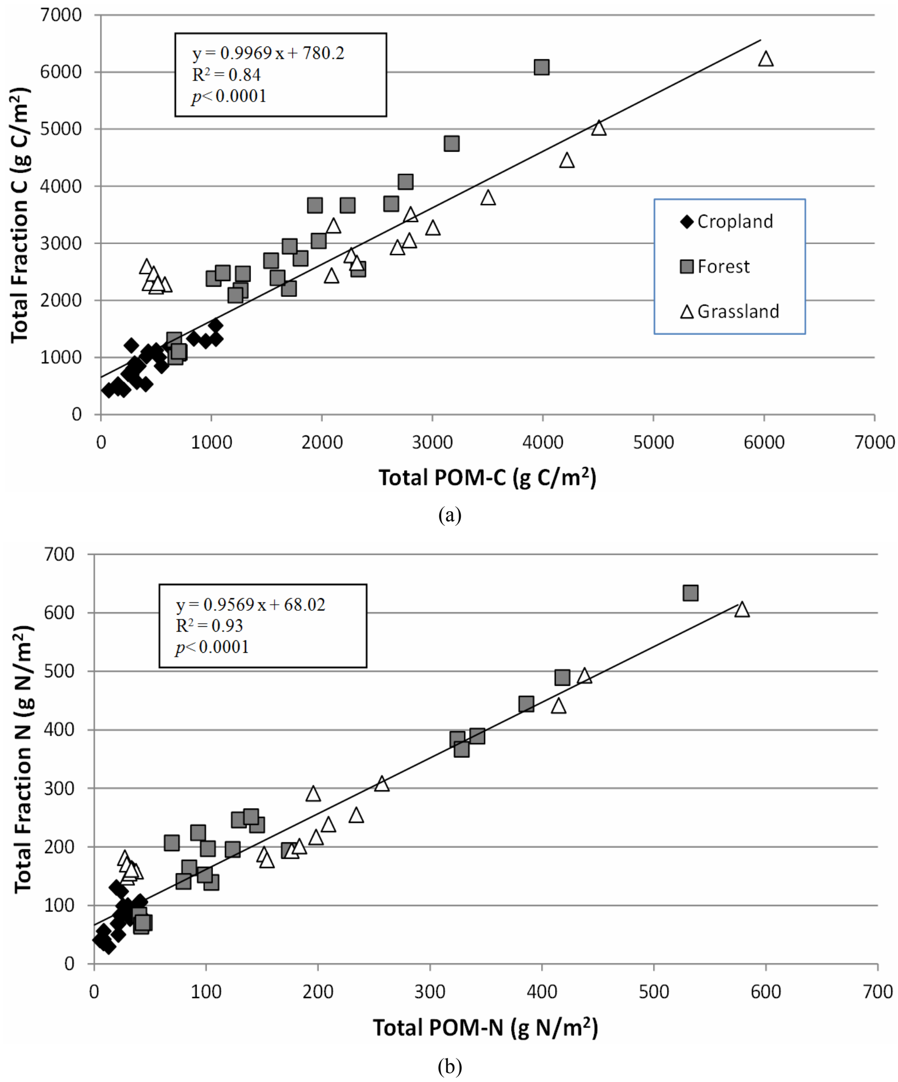



**Figure 6. Linear regression of total particulate organic matter and total fraction organic carbon (a) and total nitrogen (b), for the 0 - 5 cm depth.**

The lower concentrations of C and N in the silt and clay fractions (**Figure 5**) as well as the greater calculated saturation deficit for the croplands (**Table 7**) indicate that the croplands are further from mineral C saturation compared to the grasslands and forests. These croplands have therefore the potential to sequester additional C and N in mineral fractions if appropriately managed. The continuous physical disturbance in tilled croplands has been shown to stimulate POM decomposition due to enhanced turnover of aggregates, which in turn decreases mineral C and N stabilization compared to minimally disturbed soils [17,19]. We postulate that when these systems are managed in an improved way (less disturbance, e.g. no-tillage, cover crops, etc.), there could be an actual increase in SOC, due to physical protection of POM through aggregate formation and subsequent mineral C association, that could be stabilized in the long term.

We conclude that the applied fractionation scheme was successful in providing insights into how and to what amount C and N are stored and stabilized in the different soils and land uses included in this study. A simple chemical dispersion followed by a mechanical sieving was sufficient to obtain a highly diagnostic fraction for total soil C and N variability among widely varying ecosystems, and supports the idea that the total POM fraction is a good predictor of total SOM in these investigated ecosystems.

## 5. Conclusion

This study shows that the investigated Nitro Europe network sites store a large fraction of C and N in POM pools that are particularly vulnerable to soil disturbance such as the one caused by land use change. Best management practices that minimize soil disturbance and increase OM inputs are therefore recommended to avoid rapid losses of SOM in these systems. Rough estimates of saturation deficits suggested most forest and grassland sites to be near mineral C and N saturation, explaining the accumulation of soil C and N in the more labile POM pools, and the strong correlation between POM-C and –N and total SOC and TN. The cropland soils, on the other hand, being further from saturation, appeared to have greater potential to sequester C and N in the mineral fractions, which may be promoted under appropriate management. The observed lower C and N concentration in the silt and clay fractions points to the lower C and N stabilization efficiency at these cropland sites, likely due to the continuous physical disturbance and enhanced aggregate turnover.

## 6. Acknowledgements

Publication of this article was sponsored by the Colorado State University Libraries Open Access Research and Scholarship Fund. We thank Dr. Catherine Stewart for her insightful comments on this work. The study was funded by the European Union, Nitro Europe IP #017841. We are grateful to all the Nitro Europe level 3 site managers for their assistance during sampling.

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