

Soil organic matter fractionation as a tool for predicting nitrogen mineralization in silty arable soils

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Abstract

After decades of searching for a practical method to estimate the N mineralization capacity of soil, there is still no consistent methodology. Indeed it is important to have practical methods to estimate soil nitrogen release for plant uptake and that should be appropriate, less time consuming, and cost effective for farmers. We fractionated soil organic matter (SOM) to assess different fractions of SOM as predictors for net N mineralization measured from repacked (disturbed) and intact (undisturbed) soil cores in 14 weeks of laboratory incubations. A soil set consisting of surface soil from 18 cereal and root-cropped arable fields was physically fractionated into coarse and fine free particulate OM (coarse fPOM and fine fPOM), intra-microaggregate particulate OM (iPOM) and silt and clay sized OM. The silt and clay sized OM was further chemically fractionated by oxidation with 6% NaOCl to isolate an oxidation-resistant OM fraction, followed by extraction of mineral bound OM with 10% HF (HF-res OM). Stepwise multiple linear regression yielded a significant relationship between the annual N mineralization (kg N/ha) from undisturbed soil and coarse fPOM N (kg N/ha), silt and clay N (kg N/ha) and its C:N ratio ($R^2 = 0.80$; $P < 0.01$). The relative annual N mineralization (% of soil N) from disturbed soils was related to coarse fPOM N, HF-res OC (% of soil organic carbon) and its C:N ratio ($R^2 = 0.83$; $P < 0.01$). Physical fractions of SOM were thus found to be the most useful predictors for estimating the annual N mineralization rate of undisturbed soils. However, the bioavailability of physical fractions was changed due to the disturbance of soil. For disturbed soils, a presumed stable chemical SOM fraction was found to be a relevant predictor indicating that this fraction still contains bio-available N. The latter prompted a revision in our reasoning behind selective oxidation and extraction as tools for characterizing soil organic N quality with respect to N availability. Nonetheless, the present study also underscores the potential of a combined physical and chemical fractionation procedure for isolating and quantifying N fractions which preferentially contribute to bulk soil N mineralization. The N content or C:N ratio of such fractions may be used to predict N mineralization in arable soils.

Keywords: Chemical fractionation, nitrogen mineralization prediction, physical fractionation, silty arable loess soil, soil organic matter

Introduction

Realistic estimates of N mineralized from soil organic matter (SOM) are essential for determining the rate of N fertilizer application required to optimize crop yield and quality and to minimize adverse impacts of excessive N on the environment (Sharifi *et al.*, 2007). It is thought that a significant portion of

SOM is chemically and/or physically stabilized and resistant to microbial degradation, whereas a small part of the organic N is more labile and plays a prominent role as a source of substrate for N mineralization (Stanford & Smith, 1972; Parton *et al.*, 1987). Biologically based methods can successfully assess the labile organic N pool by incubating the soil under aerobic or anaerobic conditions at a specified temperature for a certain length of time. However they are time consuming and cannot fit into the batch-analysis techniques of soil-testing laboratories (Sharifi *et al.*, 2007).

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Reference is often made to the potential of SOM fractionation as a less time consuming and less laborious alternative and recent important advances in our knowledge of SOM composition have been achieved from physical and chemical fractionation (Olk & Gregorich, 2006). However, a successful general approach for explaining N mineralization is still lacking. For example, Schomberg *et al.* (2009) compared several chemical indices for potential mineralization but found that SOC and N content along with biological incubations were the only acceptable predictors and no single index correlated with the mineralization rate. Alternatively, physical methods can be used for uncomplexed OM (Gregorich & Janzen, 1996), primary particles (Balesdent *et al.*, 1998), particle-size-distributions (Guggenberger *et al.*, 1995) and organo-mineral complexes (Six *et al.*, 2004). Most researchers focus on the uncomplexed SOM fraction such as the light fraction, particulate SOM etc., which could be considered a readily available source of energy for the soil microbial biomass as it mainly comprises relatively easily degradable plant litter (Gregorich *et al.*, 2006). Uncomplexed OM is a possible source of N, but correlations between uncomplexed SOM and N mineralization strongly vary among soils (Gregorich *et al.*, 2006). This is likely due to the relatively large C:N ratio of uncomplexed OM, which often results in slight N immobilization rather than little or no net N release. Amongst others, Boone (1994) and Leinweber & Schulten (2002) therefore concluded that OM bound to the 'heavy' soil fraction (silt + clay) should constitute the primary source of potentially available N in various soils. Research by Leinweber & Schulten (2002) confirms that large amounts of relatively labile N-containing organic compounds (peptides, amino acids, etc.) are bound to humus and to iron- and aluminium hydroxides, but to date no experimental data are available that correlates N mineralization to such measurable fractions.

It thus appears that fractionation methodologies which cope with this heterogeneity in soil N quality by isolating and then quantifying soil N fractions, that are either microbially easily degradable, more resistant or recalcitrant, will need to be developed as alternatives for the classical chemical extractions and multitude of indices based mainly on uncomplexed OM. It seems impossible to derive one single universal N mineralization predictive model, but rather predictive models should be developed using data generated regionally. Therefore, also based on our previous work (Sleutel *et al.*, 2010) we accept that the applicability of any N mineralization predictive model, even when it includes universally relevant physicochemical soil N fraction data, will probably be limited to very specific combinations of former land use/soil/climate/current management.

Here, we evaluate a combined physical and chemical fractionation approach, which explicitly aims to isolate 'unprotected', 'physically protected', 'mineral protected' and 'recalcitrant' OM pools for its ability to isolate OM fractions

that are predictors for N mineralization capacity. To this aim we first assessed N mineralization in a set of 18 silty cropland soils with typical arable root-cereal crop based rotations. Second, both disturbed (sieved and repacked soil) as well as undisturbed soil cores were incubated in order to investigate and account for potential influences of soil macrostructure on the relation between the isolated fractions and N mineralization.

Materials and methods

Site description and soils

Eighteen cropland fields located at Heestert (Fields 1, 2, 3 and 4), Kluisbergen (Fields 5 and 6), Baugnies (Fields 7 and 8), Maulde (Fields 9 and 10), Villers-le-Bouillet (Fields 11, 12, 13 and 14), Kuttehoven (Fields 15 and 16) and Court-St-Etienne (Fields 17 and 18) which are all located in the loess belt in central Belgium, were selected for this study (Figure 1). General soil properties are given in Table 1. This area is characterized by arable farming on silt or silt loam textured soils and has an annual mean temperature of 9.8 °C and an annual mean precipitation of 780 mm (KMI, 2007). The crop rotation of the selected fields was primarily sugar beet/potatoes-winter wheat, but also fodder maize-winter wheat/barley and fodder maize monoculture. Standard mineral fertilization was practised in the selected fields. Besides, the fields received additional inputs of organic matter as pig or cattle slurry (20–25 t/ha/yr), stabilized cattle manure (8–15 t/ha/yr), and on one field green compost. Crop residues from fodder maize and potato (stubbles), sugar beets (heads + leaves), and winter wheat or winter barley (stubble + straw) were left on the field. The tillage operations of the selected fields were typical for this region

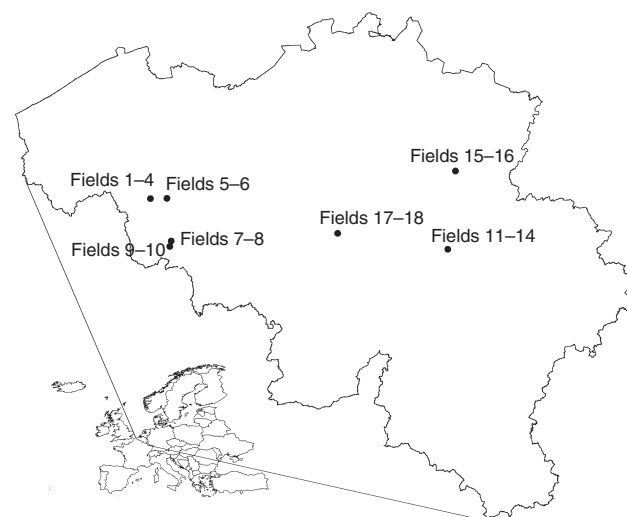


Figure 1 Location of sampling sites in Belgium.

Table 1 Selected properties of the sampled soils

Field	Soil particles (%)			OC (<2 µm)	OC (g/kg)	N (g/kg)	Bulk density (g/cm ³)	pH _{KCl}	OC _{mic} (g/kg)	OC _{HWE} (g/kg)	N _{HWE} (g/kg)	Extractable Fe and Al* (g/kg)				
	> 50 µm	2–50 µm	< 2 µm									Fe _o	Fe _p	Fe _D	Al _o	Al _p
1	33.9	52.6	13.5	10.0 ± 0.3	1.09 ± 0.11	1.38 ± 0.10	7.0 ± 0.0	0.22 ± 0.02	0.38 ± 0.08	0.054 ± 0.000	3.3 ± 0.2	8.5 ± 0.4	4.9 ± 0.0	1.1 ± 0.1	1.4 ± 0.0	4.8 ± 0.2
2	33.9	53.7	12.4	10.8 ± 0.8	1.04 ± 0.15	1.49 ± 0.08	6.5 ± 0.2	0.14 ± 0.06	0.31 ± 0.03	0.048 ± 0.001	4.1 ± 0.0	9.2 ± 0.3	5.5 ± 0.1	1.2 ± 0.2	1.6 ± 0.1	5.1 ± 0.2
3	28.0	59.9	12.1	10.8 ± 0.6	1.12 ± 0.13	1.46 ± 0.02	6.6 ± 0.5	0.13 ± 0.03	0.34 ± 0.08	0.045 ± 0.001	3.7 ± 0.0	9.1 ± 0.1	5.3 ± 0.3	1.1 ± 0.0	1.5 ± 0.0	5.0 ± 0.2
4	32.9	54.4	12.7	10.8 ± 1.2	1.01 ± 0.05	1.48 ± 0.08	6.5 ± 0.8	0.11 ± 0.02	0.29 ± 0.02	0.042 ± 0.000	3.5 ± 0.1	9.6 ± 0.1	5.4 ± 0.1	1.0 ± 0.0	1.4 ± 0.1	5.1 ± 0.4
5	30.3	51.6	18.1	12.2 ± 0.6	1.16 ± 0.02	1.37 ± 0.04	6.5 ± 0.1	0.11 ± 0.02	0.35 ± 0.01	0.044 ± 0.000	5.0 ± 0.2	12.1 ± 0.2	8.8 ± 0.3	1.1 ± 0.0	1.4 ± 0.1	8.6 ± 0.5
6	27.6	56.0	16.4	8.9 ± 1.1	0.91 ± 0.12	1.43 ± 0.04	5.5 ± 0.3	0.04 ± 0.02	0.29 ± 0.04	0.034 ± 0.000	2.6 ± 0.0	12.5 ± 0.6	3.7 ± 0.5	1.1 ± 0.0	1.4 ± 0.1	3.2 ± 0.4
7	30.4	59.0	10.6	11.5 ± 0.6	1.15 ± 0.07	1.46 ± 0.01	7.3 ± 0.2	0.09 ± 0.03	0.37 ± 0.04	0.052 ± 0.000	4.4 ± 0.7	8.9 ± 0.3	2.9 ± 0.1	1.3 ± 0.2	1.4 ± 0.0	1.4 ± 0.1
8	29.3	59.6	11.1	10.0 ± 0.3	0.95 ± 0.05	1.24 ± 0.07	6.7 ± 0.2	0.06 ± 0.01	0.30 ± 0.00	0.039 ± 0.000	3.0 ± 0.1	7.3 ± 0.0	2.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.1	0.8 ± 0.1
9	8.5	70.9	20.6	12.5 ± 0.3	1.27 ± 0.10	1.43 ± 0.04	6.1 ± 0.1	0.27 ± 0.04	0.38 ± 0.04	0.051 ± 0.000	3.7 ± 0.1	6.4 ± 0.2	2.8 ± 0.0	1.1 ± 0.2	1.7 ± 0.0	1.5 ± 0.0
10	8.5	77.6	13.9	10.8 ± 0.3	1.12 ± 0.02	1.49 ± 0.04	5.7 ± 0.1	0.15 ± 0.01	0.31 ± 0.01	0.044 ± 0.000	3.7 ± 0.3	8.6 ± 0.3	3.5 ± 0.3	1.1 ± 0.3	1.2 ± 0.0	2.3 ± 0.1
11	7.9	72.2	19.8	16.1 ± 1.5	1.55 ± 0.11	1.37 ± 0.04	6.5 ± 0.2	0.08 ± 0.01	0.57 ± 0.02	0.079 ± 0.000	3.6 ± 0.2	13.6 ± 0.1	3.5 ± 0.7	1.4 ± 0.1	1.9 ± 0.1	1.1 ± 0.1
12	5.7	75.4	18.9	11.1 ± 0.4	1.28 ± 0.08	1.40 ± 0.3	6.6 ± 0.1	0.10 ± 0.01	0.47 ± 0.03	0.064 ± 0.000	3.6 ± 0.1	12.4 ± 0.6	2.2 ± 0.8	1.4 ± 0.0	1.7 ± 0.1	0.9 ± 0.0
13	6.1	77.2	16.2	13.9 ± 1.4	1.39 ± 0.02	1.47 ± 0.3	6.5 ± 0.1	0.09 ± 0.02	0.39 ± 0.01	0.062 ± 0.000	3.6 ± 0.0	10.6 ± 0.0	2.3 ± 0.4	1.3 ± 0.0	1.6 ± 0.0	0.9 ± 0.1
14	9.4	74.6	16.2	9.4 ± 0.2	0.97 ± 0.02	1.49 ± 0.04	5.8 ± 0.1	0.06 ± 0.01	0.28 ± 0.01	0.033 ± 0.000	3.5 ± 0.0	10.0 ± 0.3	2.7 ± 1.1	1.2 ± 0.0	1.4 ± 0.0	0.8 ± 0.0
15	12.8	71.7	15.5	13.0 ± 0.8	1.18 ± 0.10	1.47 ± 0.01	5.8 ± 0.1	0.05 ± 0.01	0.39 ± 0.02	0.043 ± 0.000	3.4 ± 0.2	9.9 ± 0.5	3.0 ± 0.0	1.1 ± 0.1	1.4 ± 0.2	1.7 ± 0.0
16	11.1	71.5	17.4	9.7 ± 0.2	1.10 ± 0.01	1.51 ± 0.02	6.4 ± 0.2	0.07 ± 0.04	0.30 ± 0.01	0.048 ± 0.000	3.2 ± 0.2	5.8 ± 0.3	3.0 ± 0.8	1.1 ± 0.0	1.5 ± 0.1	1.4 ± 0.2
17	13.8	71.5	14.7	11.3 ± 1.3	1.17 ± 0.07	1.35 ± 0.08	6.3 ± 0.2	0.08 ± 0.02	0.27 ± 0.00	0.036 ± 0.000	3.7 ± 0.1	11.4 ± 0.3	2.8 ± 0.1	1.3 ± 0.0	1.6 ± 0.0	1.3 ± 0.1
18	8.2	75.7	16.0	9.2 ± 0.4	0.99 ± 0.02	1.47 ± 0.08	6.0 ± 0.3	0.04 ± 0.01	0.30 ± 0.01	0.047 ± 0.001	3.5 ± 0.0	11.7 ± 0.4	2.2 ± 0.0	1.2 ± 0.0	1.5 ± 0.1	1.0 ± 0.0

*Fe_o, Al_o = Ammonium oxalate-extractable Fe and Al; Fe_D, Al_D = Na-dithionite-citrate-bicarbonate-extractable Fe and Al; Fe_p, Al_p = Na-pyrophosphate-extractable Fe and Al.

covering both non-inversion tillage with a cultivator or soil loosener (RTC) (10–15 cm), and direct drilling with rotary harrow (5 cm) next to inversion tillage with mouldboard plough (25–30 cm). The crop rotation and management operations of the selected fields are explained in detail in D'Haene *et al.* (2008a,b). Soil samples were collected from three subplots of 150 m² (10 × 15 m) per field, each spaced 10 m apart from each other. To avoid edge effects, the subplots were located more than 20 m apart from the edges of the fields. For each subplot 15 samples were collected from the surface soil (0–10 cm) in March 2005 by means of an auger (Ø 2.5 cm) and these samples were bulked. Part of the field moist soil was gently broken apart by hand and was passed through an 8-mm sieve to break down large macroaggregates. The soil was then dried at 50 °C. Three replicates of 10 g sub-samples were used for physical fractionation.

Physical fractionation of soil organic matter

Organic matter in bulked whole soil samples was physically fractionated into OM associated with silt and clay particles, into particulate organic matter POM residing in stable microaggregates and into the free particulate organic matter (fPOM) based on the microaggregate isolation method proposed by Six *et al.* (2002) and as described by Sleutel *et al.* (2006, 2007). This physical fractionation procedure results in the sequential isolation of (1) water-stable microaggregates from coarse free particulate organic matter (coarse fPOM) (>250 µm) and silt and clay sized OM (<53 µm) by means of wet sieving and (2) density separation of fine fPOM (53–250 µm) from intra-microaggregate POM (iPOM) and intra-microaggregate silt and clay associated OM (<53 µm) and (3) isolation of iPOM (53–250 µm) from the latter OM fraction (Figure 2).

Chemical fractionation of SOM

The silt and clay fraction (<53 µm) was then subjected to a chemical fractionation procedure, according to Mikutta *et al.* (2006) and slightly modified by Sleutel *et al.* (2009), which involves sequential oxidation with 6% NaOCl and mineral extraction with 10% HF-acid. The procedure results in the isolation of (1) a chemically stable 6% NaOCl resistant SOM fraction composed of mineral-protected as well as biochemically recalcitrant OM and (2) a biochemically non-bound recalcitrant SOM fraction (Figure 2). A ball-milled 5 g silt and clay sized sample was reacted three times for 6 h with 50 mL 6% NaOCl adjusted to pH 8.0 inside 85 mL nalgene centrifuge tubes. Samples were centrifuged and decanted between oxidation cycles and were ultimately washed once with 1 M NaCl and three times with deionized H₂O. After drying and weighing, a sub sample was used for total C and N analysis. Then, 3 g of the oxidation residue was treated

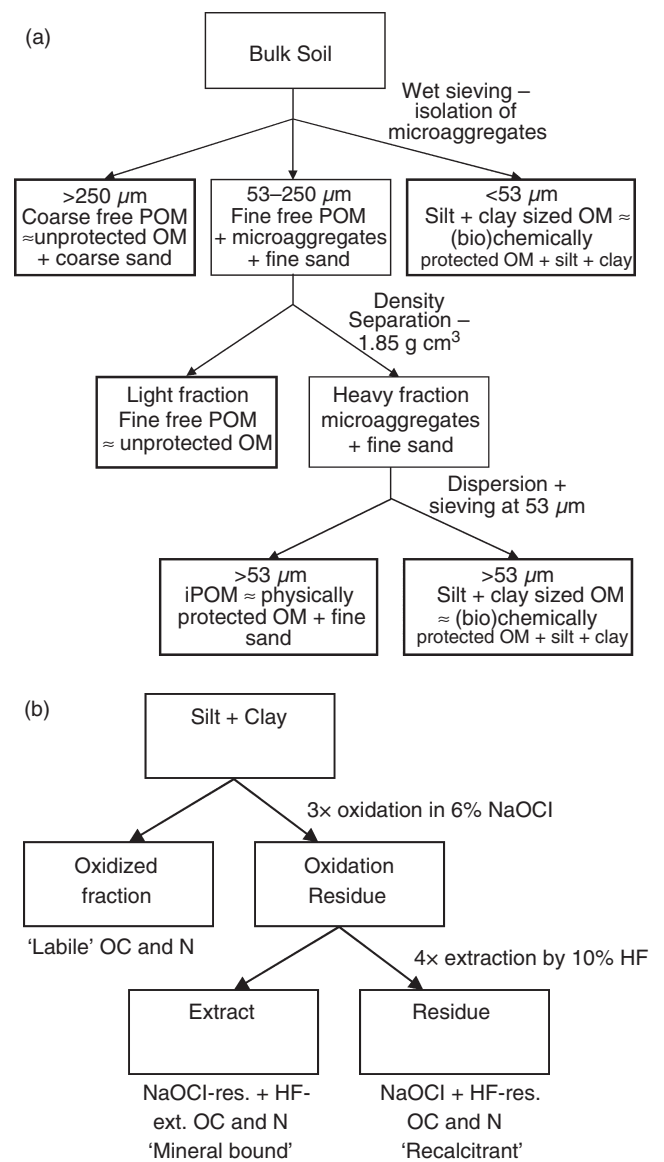


Figure 2 (a) The physical fractionation method used for the separation of coarse free particulate organic matter (coarse fPOM), fine free POM (fine fPOM), intra-microaggregate POM (iPOM) and silt and clay sized OM, with indication of the isolated conceptual soil organic matter (SOM) pools according to the model of Six *et al.* (2002) (unprotected OM, physically protected OM, OM protected through mineral association or biochemical recalcitrance). (b) chemical fractionation procedure used for the separation of a 6% NaOCl oxidizable fraction, a 6% NaOCl treatment resistant but 10% HF-extractable fraction and the 6% NaOCl and subsequent 10% HF treatment residues, with indication of the isolated conceptual SOM pools according to Mikutta *et al.* (2006) (Labile OM, mineral protected OM and recalcitrant OM).

four times with 20 mL 10% HF in order to dissolve and remove mineral constituents and mineral-bound OM. Extraction residues were washed five times with deionized

H₂O to remove salts and residual HF and were dried and weighed. Chemical fractionations were carried out twice.

Microbial biomass C and hot-water extractable OM

Soil microbial biomass OC (OC_{mic}) was determined by the fumigation–extraction method (Vance *et al.*, 1987). K₂SO₄ extracts before and after fumigation were filtered through paper filters (Whatman 5). The K₂SO₄ extracts were analyzed for dissolved OC (TOC analyzer, Shimadzu, Japan). Extraction with hot aqueous solutions was used to measure labile OC (OC_{HWE}) based on a procedure by Leinweber *et al.* (1995). 10 g of dry soil was weighed into nalgene centrifuge tubes. 50 mL of distilled water was added to the tubes and these tubes were capped and left for 60 min in a hot water bath at 100 °C. The tubes were centrifuged for 20 min at 850 g and the supernatants were filtered through Whatman (GF/C) glass microfibre filters and stored in vials (Ghani *et al.*, 2003). The extracts were analyzed for OC concentration with a Shimadzu TOC analyzer and total N was determined after alkaline persulphate oxidation (Koroleff, 1983) as NO₃⁻ with a continuous flow autoanalyzer.

Nitrogen mineralization

N mineralization was measured under controlled conditions at 15 °C in the laboratory on undisturbed and disturbed soil samples for a period of 14 weeks. PVC tubes with a 4 cm inner diameter and 18 cm height were used as incubation containers. The soil sampling protocol for disturbed and undisturbed N mineralization of the selected fields is described in detail by D'Haene *et al.* (2008a). Basically 14 PVC tubes (Ø 4 cm, height 18 cm) were pushed into the soil and dug out carefully from each sub-plot (three sub-plots per field, totalling 756 tubes). Seven tubes per sub-plot were incubated 'undisturbed'. The soil from the other seven tubes was removed and the tubes were then refilled with the 'disturbed soil', adjusted to the same bulk density. Every 2 weeks soils were sampled destructively by removing the soil from one tube for each sub-plot. The soil of each tube was mixed thoroughly and 30 g moist soil was analyzed for mineral N (NO₃⁻-N and NH₄⁺-N) by extraction with a 1 M KCl [1:2, soil weight (g):extractant volume (mL)] solution. The mineral N concentration in the extract was measured colorimetrically with a 'continuous flow auto-analyzer' (Chemlab System 4, Skalar, the Netherlands). N mineralization rates were estimated by fitting a zero-order model: $N_t = N_0 + k \cdot t$, where t is the time (in days), N_t is the amount of mineral N at time t , N_0 is the initial amount of mineral N, and k is the mineralization rate (mg N/kg dry soil/day). A temperature correction function by De Neve *et al.* (1996) was used to recalculate the N mineralization rate obtained in the laboratory to N mineralization per ha per year under field conditions (k_{field}) (kg N/ha/yr) using

the yearly average temperature. A relative yearly soil N turnover rate (k_{turn}) (% of total N/yr) was calculated by dividing k_{field} by the N stock.

Carbon and nitrogen analysis

Sub samples (200–800 mg) of the soil samples and all physical and chemical fractions of the soils that contain no CaCO₃ were analyzed for total C content by dry combustion with a Variomax CNS-analyzer (Elementar Analysesysteme, Germany). Original soil and sub samples (200 mg) of the iPOM fraction + fine sand and the silt + clay (< 53 µm) fractions of the soils containing CaCO₃ were analyzed for both OC and inorganic carbon (IC) content with a Shimadzu TOC analyzer. The soil organic nitrogen (SON) content was determined in all the fractions by dry combustion (Variomax CNS-analyzer, Elementar Analysesysteme, Germany) assuming that the mineral N fractions (NH₄⁺ and NO₃⁻) were dissolved with decanted water. Absolute amounts of C and N in the different fractions expressed per grams of soil were calculated from the relative dry matter weight of each fraction and its percentage of C and N.

Statistical analysis

Correlation and multivariate linear regression analysis were used to investigate the relation between the isolated physical and chemical fractions and the annual N mineralization rate with the SPSS 15.0 package (SPSS Inc., USA). For the multivariate regression analysis, a stepwise linear regression was used considering the absolute (k_{field}) and relative (k_{turn}) annual N mineralization rate as dependents and absolute (SOC and N in kg/ha) amounts and relative (in % of SOC and soil N) proportions of SOC and N over the isolated physical and chemical fractions and other soil parameters as predictor variables. The successive selection of independent in the final regression model herein takes the form of a sequence of F-tests in which the independent variables are successively entered (if $F < P\text{-in} = 0.05$) or removed (if $F > P\text{-out} = 0.10$).

Results

N mineralization

The evolution of mineral N (NO₃⁻-N + NH₄⁺-N) both in disturbed and undisturbed soil cores for field 12 is given as an example in Figure 3. The amount of mineral N (NO₃⁻-N + NH₄⁺-N) increased linearly with time. However, large variability between replicates was observed (Table 2). The N mineralization measured from undisturbed soil samples was between 7 and 82 kg N/ha/yr and accounted for 0.5 to 4.7% of the soil N (Table 2). N mineralization measured from undisturbed soil samples was between 12 and 90 kg N/ha/yr

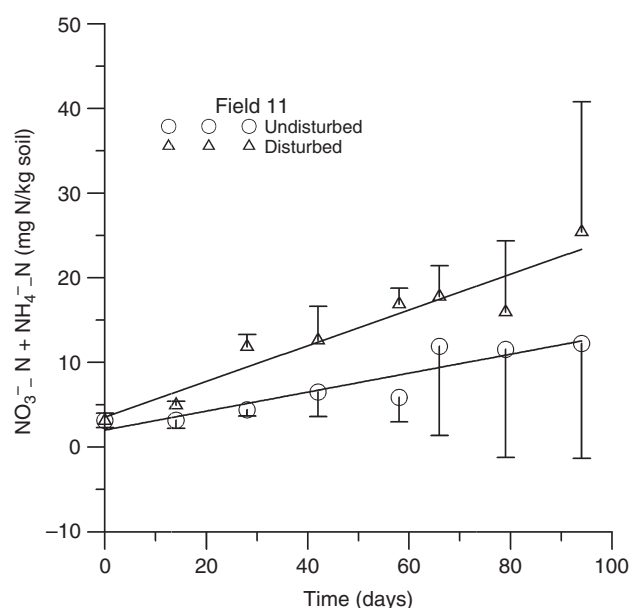


Figure 3 Evolution of mineral N ($\text{mg NH}_4^+\text{-N} + \text{NO}_3^-\text{-N/kg}$ dry soil) (line = standard deviation) in soil (Field 11) incubated under both disturbed and undisturbed conditions.

and accounted for 0.8 to 5.0% of the soil N (Table 2). These rates are in the same order of magnitude as yearly N mineralization of 19 to 67 kg N/ha (0–10 cm) and turnover rates of 1.80 to 3.41% of soil N reported by Anonymous (2002) for 10 Belgian silty arable soils. Over all 18 soils, a significantly higher N mineralization ($P < 0.01$, paired

samples *t*-test) was observed in the disturbed (43.7 ± 25.9 kg N/ha/yr and $2.6 \pm 1.3\%$ of the soil N) compared with the undisturbed soil cores (33.7 ± 21.4 kg N/ha/yr and $2.0 \pm 1.2\%$ of the soil N).

Physical N fractions

Four different N fractions were isolated by physical fractionation, namely N in coarse fPOM, fine fPOM, iPOM and silt and clay N. The coarse fPOM N and fine fPOM N account for 2.2 to 6.4% and 0.6 to 3.4% of soil N, respectively (Table 3). High C:N ratios of both fine and coarse free POM (on average 22 and 24 respectively) with large variability reflect the presence of undecomposed or partially decomposed OM. The iPOM N range between 96 and 323 kg N/ha and constitute a somewhat larger share of the soil N than fPOM (on average 4.4 to 9.4% of total N). The C:N ratio of the iPOM fraction (on average 15) was lower than of the two fPOM fractions, but higher than of the silt and clay size fraction (average 8.3), which contained the largest amount of soil N (82 to 92% of soil N), ranging between 949 and 1905 kg N/ha.

OC_{HWE} and N_{HWE} represent 3% to 5% of SOC and 2% to 5% of soil N, in line with 3% to 5% OC_{HWE} and N_{HWE} found by Leinweber *et al.* (1995) and 2% to 7.5% N_{HWE} found by Curtin *et al.* (2006).

Chemical N fractions

The size of the N fractions obtained from the chemical fractionation of silt and clay sized OM, expressed on a bulk

Table 2 Annual N mineralization rate (k_{field}) and annual N turnover rate (k_{turn}) recalculated to the average annual field temperature

Field	k_{field} (kg N/ha/yr)		k_{turn} (% of total N/yr)	
	Undisturbed soil	Disturbed soil	Undisturbed soil	Disturbed soil
1	23.79 ± 6.49	13.57 ± 2.08	1.58 ± 0.43	0.90 ± 0.14
2	23.74 ± 3.57	16.34 ± 6.86	1.53 ± 0.23	1.05 ± 0.44
3	18.17 ± 12.27	12.71 ± 5.11	1.11 ± 0.75	0.78 ± 0.31
4	18.57 ± 10.19	22.25 ± 12.98	1.24 ± 0.68	1.49 ± 0.54
5	9.44 ± 5.46	21.99 ± 12.29	0.59 ± 0.34	1.38 ± 0.77
6	15.21 ± 1.81	15.51 ± 6.44	1.17 ± 0.14	1.19 ± 0.49
7	21.86 ± 11.78	34.48 ± 1.10	1.38 ± 0.75	2.18 ± 0.07
8	7.23 ± 5.04	29.43 ± 21.63	0.50 ± 0.35	2.02 ± 1.49
9	39.11 ± 11.21	46.60 ± 15.16	2.33 ± 0.67	2.78 ± 0.90
10	33.39 ± 11.85	35.85 ± 14.66	2.83 ± 1.01	3.04 ± 1.24
11	24.08 ± 17.52	60.32 ± 36.57	1.13 ± 0.77	2.84 ± 1.72
12	60.12 ± 24.01	57.05 ± 23.25	3.35 ± 1.34	3.18 ± 1.30
13	65.31 ± 21.49	89.79 ± 19.05	3.20 ± 1.05	4.39 ± 0.93
14	44.08 ± 5.77	53.01 ± 10.23	3.05 ± 0.40	3.67 ± 0.71
15	81.84 ± 17.02	86.12 ± 20.36	4.72 ± 0.98	4.97 ± 1.17
16	28.05 ± 2.78	52.62 ± 9.36	1.69 ± 0.17	3.17 ± 0.56
17	66.26 ± 5.70	89.96 ± 11.56	3.65 ± 0.31	4.95 ± 0.64
18	26.97 ± 6.66	49.46 ± 8.94	1.62 ± 0.40	2.96 ± 0.54

Table 3 Amounts of total N (kg N/ha) in and C:N ratios of the isolated soil physical fractions (average \pm standard deviation)

Field	Particulate organic matter (POM)							
	Coarse fPOM (> 250 μ m)		Inter-micro-aggregate fine fPOM (53–250 μ m)		Intra-micro-aggregate POM (53–250 μ m)		Silt + clay (< 53 μ m)	
	N	C:N	N	C:N	N	C:N	N	C:N
1	91 \pm 32	30.4 \pm 8.3	37 \pm 7	26.4 \pm 2.3	131 \pm 22	16.5 \pm 0.8	1352 \pm 87	8.6 \pm 0.8
2	126 \pm 9	27.3 \pm 9.6	45 \pm 18	24.8 \pm 10.6	144 \pm 12	15.2 \pm 0.3	1408 \pm 68	8.6 \pm 0.5
3	108 \pm 43	25.1 \pm 4.9	43 \pm 8	23.2 \pm 2.5	174 \pm 86	14.5 \pm 0.9	1385 \pm 96	8.8 \pm 0.3
4	72 \pm 14	27.1 \pm 2.3	42 \pm 9	25.2 \pm 1.1	104 \pm 12	15.5 \pm 2.4	1245 \pm 61	8.7 \pm 0.4
5	90 \pm 18	21.5 \pm 3.7	56 \pm 17	17.8 \pm 1.8	159 \pm 9	14.4 \pm 0.5	1244 \pm 57	8.9 \pm 0.7
6	59 \pm 8	16.1 \pm 1.5	32 \pm 7	19.0 \pm 1.0	96 \pm 6	13.4 \pm 0.5	949 \pm 52	8.7 \pm 0.9
7	72 \pm 13	19.5 \pm 2.9	40 \pm 10	19.1 \pm 1.4	96 \pm 0	18.0 \pm 0	1183 \pm 96	8.1 \pm 0.8
8	73 \pm 17	21.1 \pm 7.6	40 \pm 20	15.8 \pm 3.8	101 \pm 2	14.8 \pm 2.6	1136 \pm 47	8.7 \pm 0.1
9	152 \pm 32	23.4 \pm 8.9	84 \pm 17	16.1 \pm 0.9	185 \pm 20	13.8 \pm 0.9	1317 \pm 84	9.3 \pm 0.5
10	38 \pm 9	30.9 \pm 2.2	34 \pm 7	24.3 \pm 0.9	115 \pm 7	15.5 \pm 0.5	1079 \pm 52	8.1 \pm 0.3
11	175 \pm 21	19.3 \pm 2.9	25 \pm 10	30.0 \pm 5.2	323 \pm 27	14.7 \pm 0.9	1905 \pm 63	8.0 \pm 0.7
12	118 \pm 13	19.9 \pm 4.5	47 \pm 2	17.7 \pm 0.6	162 \pm 19	14.3 \pm 0.7	1464 \pm 28	7.5 \pm 0.2
13	157 \pm 39	22.9 \pm 8.4	52 \pm 8	18.7 \pm 1.0	227 \pm 6	13.1 \pm 0.2	1797 \pm 49	7.4 \pm 0.2
14	109 \pm 65	32.8 \pm 7.8	19 \pm 14	20.7 \pm 3.8	154 \pm 26	12.3 \pm 0.4	1436 \pm 66	7.4 \pm 0.4
15	174 \pm 30	20.4 \pm 2.6	28 \pm 4	30.4 \pm 3.9	263 \pm 18	13.4 \pm 0.1	1449 \pm 31	7.9 \pm 0.6
16	72 \pm 22	20.5 \pm 2.5	25 \pm 11	27.9 \pm 6.3	160 \pm 25	14.0 \pm 0.5	1449 \pm 40	7.8 \pm 0.1
17	195 \pm 19	23.7 \pm 2.9	81 \pm 9	19.4 \pm 2.3	178 \pm 73	14.7 \pm 1.0	1421 \pm 48	8.1 \pm 0.6
18	121 \pm 47	31.2 \pm 4.4	54 \pm 22	23.1 \pm 1.6	144 \pm 29	13.7 \pm 1.7	1392 \pm 46	8.3 \pm 0.4

fPOM, free particulate organic matter.

Fields	NaOCl-resistant OM					
	NaOCl-oxidizable OM (NaOCl-ox)		Extracted by HF OM (HF-ext)		HF-resistant OM (HF-res)	
	N	C:N	N	C:N	N	C:N
1	735 \pm 58	6.9 \pm 1.5	333 \pm 95	5.9 \pm 2.2	284 \pm 38	16.3 \pm 1.2
2	818 \pm 39	6.3 \pm 0.3	355 \pm 14	8.0 \pm 1.9	235 \pm 25	17.8 \pm 0.2
3	817 \pm 2	6.7 \pm 0.3	327 \pm 14	7.9 \pm 0.0	241 \pm 16	17.3 \pm 0.2
4	594 \pm 102	6.1 \pm 0.5	371 \pm 116	7.5 \pm 1.8	279 \pm 14	16.1 \pm 1.2
5	556 \pm 47	6.2 \pm 2.3	389 \pm 45	8.1 \pm 1.9	299 \pm 2	14.8 \pm 0.1
6	370 \pm 83	6.7 \pm 1.9	369 \pm 94	6.4 \pm 2.2	210 \pm 11	16.4 \pm 1.8
7	635 \pm 7	5.6 \pm 0.0	315 \pm 12	7.7 \pm 0.4	233 \pm 6	15.6 \pm 0.0
8	546 \pm 116	5.8 \pm 1.0	364 \pm 129	7.6 \pm 2.5	227 \pm 14	17.7 \pm 0.8
9	652 \pm 14	7.5 \pm 1.4	347 \pm 53	8.7 \pm 1.4	319 \pm 67	13.4 \pm 1.9
10	555 \pm 29	5.4 \pm 0.7	235 \pm 69	9.5 \pm 2.7	289 \pm 40	12.2 \pm 1.2
11	1000 \pm 122	5.6 \pm 0.2	421 \pm 130	9.0 \pm 0.8	484 \pm 8	12.0 \pm 0.4
12	846 \pm 29	4.9 \pm 0.1	192 \pm 2	11.6 \pm 1.1	426 \pm 28	10.8 \pm 0.8
13	1001 \pm 51	5.2 \pm 0.5	372 \pm 19	6.0 \pm 1.0	424 \pm 70	13.8 \pm 1.2
14	811 \pm 115	5.1 \pm 0.4	129 \pm 78	17.5 \pm 6.1	495 \pm 37	8.5 \pm 0.5
15	687 \pm 56	5.0 \pm 0.0	338 \pm 71	10.3 \pm 1.7	424 \pm 15	10.7 \pm 0.5
16	797 \pm 150	5.4 \pm 1.0	254 \pm 144	10.1 \pm 2.0	398 \pm 6	11.2 \pm 0.0
17	729 \pm 54	6.0 \pm 0.2	299 \pm 19	7.6 \pm 1.4	392 \pm 35	12.4 \pm 0.5
18	764 \pm 27	6.2 \pm 0.8	249 \pm 18	10.4 \pm 2.2	379 \pm 9	11.1 \pm 0.4

OM, organic matter.

Table 4 Amounts of total N (kg N/ha) and C:N ratios of the isolated chemical fractions (average \pm standard deviation)

soil basis (kg/ha soil), is given in Table 4. Briefly, the NaOCl oxidation removed on average $52 \pm 5.4\%$ of the silt and clay size N (i.e. $45 \pm 4.8\%$ of soil N). This result corresponds with Kleber *et al.* (2005) and Sleutel *et al.* (2009) who found $55 \pm 26\%$ and $64 \pm 19\%$ NaOCl resistant N (NaOCl-res N) in different soil types. N-containing OM constituents were preferentially removed as the calculated C:N ratio of the NaOCl oxidized (NaOCl-ox) OM (5.9 ± 0.7) was lower than of the silt and clay OM (8.3 ± 0.6). Subsequent mineral dissolution by 10% HF reduced the sample dry matter by 58 ± 3 wt% and released on average $48 \pm 12\%$ of the NaOCl-res N, much less than the previously reported losses of $67 \pm 12\%$ by Sleutel *et al.* (2009) and $87 \pm 7\%$ of NaOCl-res N by Mikutta *et al.* (2006), with a C:N ratio of 8.9 ± 2.7 . The percentage of subsequent HF resistant residual N (HF-res N) ($21.1 \pm 4.4\%$) was found to be slightly larger than the HF extractable N (HF-ext N) ($20.3 \pm 6\%$) ranging between 210 and 595 kg N/ha with an average C:N ratio of 13.8 ± 2.8 .

Correlation between N mineralization, soil properties and N fractions

Pearson's correlation coefficients between the absolute (k_{field}) and relative (k_{turn}) annual N mineralization rate and soil properties and absolute (kg/ha) and relative (%) amounts of SOC and N in the physical and chemical fractions are listed in Tables 5 and 6, respectively. Gallagher & Bartholomew (1964) found that predictions of N availability were improved when N mineralization and its predictive indices were

Table 5 Pearson correlation coefficients (r) between the annual N mineralization rate (k_{field}) and annual N turnover rate (k_{turn}) and general soil properties

Soil parameters	k_{field} (kg N/ha/yr)		k_{turn} (% of total N/yr)	
	Undisturbed soil	Disturbed soil	Undisturbed soil	Disturbed soil
Total N	0.20	0.31	0.20	0.31
Total C	0.17	0.20	0.17	0.20
C:N ratio	0.00	-0.19	0.00	-0.19
%Sand	-0.64**	-0.76**	-0.61**	-0.74**
%Silt	0.66**	0.77**	0.65**	0.78**
%Clay	0.29	0.39	0.23	0.31
Fe _o	-0.18	-0.11	-0.18	-0.11
Fe _D	-0.28	-0.23	-0.28	-0.23
Fe _P	-0.52*	-0.63**	-0.52*	-0.63**
Al _o	0.06	0.17	0.06	0.17
Al _D	-0.02	0.09	-0.02	0.09
Al _P	-0.51*	-0.67**	-0.51*	-0.67**
pH	-0.38	-0.31	-0.38	-0.31

Correlation is significant with ** $P = 0.01$ (two-tailed) and * $P = 0.05$ (two-tailed).

combined in multiple regressions. Therefore, a stepwise linear regression was conducted between k_{field} measured under disturbed and undisturbed conditions (Table 2) and the amount of OC and N in all the isolated physical and

Table 6 Pearson correlation coefficients between annual N mineralization rate (k_{field}) and amounts (kg N/ha) of physical and chemical fractions of SOC and N (left) and annual N turnover rate (k_{turn}) and the proportion (%) of SOC and N) of physical and chemical fractions of SOC and N (right)

SOC and N fractions	k_{field} (kg N/ha/yr)		k_{turn} (% of total N/yr)	
	Undisturbed soil	Disturbed soil	Undisturbed soil	Disturbed soil
N _{HWE}	0.13	0.26	0.28	0.16
OC _{HWE}	0.23	0.30	-0.31	-0.34
C:N ratio OC _{HWE}	0.19	0.05	-0.11	-0.10
OC _{mic}	-0.12	-0.33	-0.17	-0.41
Coarse fPOM N	0.66**	0.72**	0.58*	0.59**
Coarse fPOM C	0.58*	0.59*	0.52*	0.51*
C:N ratio coarse fPOM	-0.02	-0.16	0.02	-0.12
Fine fPOM N	0.18	0.18	0.02	0.04
Fine fPOM C	0.22	0.18	0.05	0.01
C:N ratio fine fPOM	0.09	0.07	0.08	0.01
iPOM N	0.50*	0.63**	0.48*	0.51*
iPOM C	0.42	0.55*	0.37	0.46
C:N ratio iPOM	-0.38	-0.42	-0.34	-0.39
POM N	0.61**	0.71**	0.57*	0.60**
POM C	0.57*	0.63**	0.61**	0.62**
C:N ratio POM	-0.12	-0.27	-0.09	-0.25
Silt and clay N	0.44	0.61**	0.28	0.35
Silt and clay C	0.22	0.37	-0.40	-0.23
C:N ratio silt and clay SOM	-0.62**	-0.68**	-0.57*	-0.64**
NaOCl-ox N	0.40	0.48*	0.20	0.14
NaOCl-ox OC	0.12	0.16	-0.34	-0.38
C:N ratio NaOCl-ox SOM	-0.48*	-0.56*	-0.46	-0.58*
NaOCl-res N	0.39	0.63**	-0.04	0.04
NaOCl-res OC	0.26	0.48*	-0.22	-0.04
C:N ratio NaOCl-res SOM	-0.46	-0.57*	-0.45	-0.55*
HF-ext N	-0.31	-0.18	-0.48*	-0.46
HF-ext OC	-0.06	0.11	-0.28	-0.15
C:N ratio HF-ext SOM	0.27	0.25	0.33	0.32
HF-res N	0.65**	0.78**	0.60**	0.67**
HF-res OC	0.41	0.60**	-0.09	0.10
C:N ratio HF-res SOM	-0.62**	-0.69**	-0.63**	-0.72**

iPOM, intra-microaggregate particulate OM; SOM, soil organic matter. Correlation is significant with ** $P = 0.01$ (two-tailed) and * $P = 0.05$ (two-tailed).

chemical fractions including their C:N ratios. This analysis yielded the following two models:

$$k_{\text{field};\text{undisturbed}} = 384 + 0.47 \text{ Coarse fPOM N} \\ - 38.1 (\text{Silt} + \text{Clay C:N}) - 0.073 (\text{Silt} + \text{Clay N}) \\ + 0.084 \text{ OC}_{\text{mic}} \quad (R^2 = 0.87; P < 0.01, N = 18) \quad (1)$$

$$k_{\text{field};\text{disturbed}} = 126 + 0.15 (\text{HF-res N}) \\ + 0.48 \text{ Coarse fPOM N} - 0.02 (\text{NaOCl-ox C}) \\ - 13.7 \text{ C:N}_{\text{HWE}} \quad (R^2 = 0.89; P < 0.01, N = 18). \quad (2)$$

Next a stepwise linear regression was conducted between k_{turn} (Table 2) and the relative proportions of SOC and SN of all the isolated fractions (in % of SOC or N) along with their C:N ratios.

$$k_{\text{turn};\text{undisturbed}} = 3.42 - 0.22 \text{ C:N}_{\text{Recalcitrant OM}} \\ + 0.25\% \text{ Coarse fPOM N} \quad (3) \\ (R^2 = 0.57; P < 0.01, N = 18)$$

$$k_{\text{turn};\text{disturbed}} = 0.38 - 0.31 \text{ C:N}_{\text{Recalcitrant OM}} \\ + 0.46\% \text{ Coarse fPOM N} + 0.24 (\text{HF-res OC}) \\ - 0.5 \text{ C:N}_{\text{HWE}} \quad (R^2 = 0.88; P < 0.01, N = 18). \quad (4)$$

Coarse fPOM N and C:N ratios of silt and clay N fractions were found equally important in predicting undisturbed k_{field} in model 1 followed by silt and clay N and OC_{mic} . Coarse fPOM N was also found as the highest contributor in predicting disturbed k_{field} followed by HF-res N, NaOCl-ox C and C:N ratio OC_{HWE} in model 2. However for model 3, the C:N ratio of HF-res OM was the best predictor. Percent coarse fPOM N was found to be the largest contributor in predicting disturbed k_{turn} in model 4 followed by the C:N ratios of HF-res OM, % HF-res OC and C:N ratio of OC_{HWE} . Model prediction for N mineralization both under disturbed and undisturbed condition agreed well with those of the observed values (Figure 4).

Discussion

In spite of strong similarities in crop rotation (root crops-cereals), fertilization (manuring mostly cattle slurry + standard mineral NPK fertilization) and soil texture (silty soils), there was a relative large variation in N mineralization and in the distribution of OC and N over the isolated fractions. The applied fractionation approach was selected to separate OC and N into fractions, which at least to some extent coincide with SOM pools that are subjected to differing degrees of physicochemical stabilization. Firstly, according to Six *et al.* (2002) coarse and fine fPOM, iPOM and silt and clay sized SOM fraction represent an unprotected, physically protected and (bio)-chemically

protected SOM pools, respectively. This assumption seems simplistic as many studies have found the silt and clay fraction to contain labile SOM constituents as well, consisting of, for example, soil microbial biomass and its metabolites and root exudates and lysates (e.g. Leinweber & Schulten, 1995; Christensen, 2001). A further subdivision of the silt and clay sized OM into labile, mineral bound, and recalcitrant presumed OM pools was obtained through sequential chemical fractionation following Mikutta *et al.* (2006).

For neither under disturbed or undisturbed conditions is there correlation between k_{field} and k_{turn} and SOC or soil N content (Table 5). Often, N mineralization correlates with SOC or soil N content and based on this relationship field N mineralization has been estimated (e.g. Camargo *et al.*, 2004; Sharifi *et al.*, 2007; Benintende *et al.*, 2008). The absence of a positive relation with soil N content suggests that other factors such as availability of water and air or substrate for microbes, or that a specific fraction of the organic N or both controlled the N mineralization of these soils. In spite of limited variation in soil texture in the studied soil set, a positive correlation was observed between k_{field} and k_{turn} and %silt and a significant negative correlation with %sand. Binding of OM to sand or silt is most likely limited and these correlations might reflect a secondary control by soil physical properties (aeration, water holding capacity) on OM decomposition. Sleutel *et al.* (2008) found that in wet silty soils under grassland, forest and shrubland soil in this study area, the bulk density was the single best predictor for N mineralization from undisturbed soil cores.

There is no correlation between k_{field} and k_{turn} and OC_{HWE} and the C:N ratio of the hot water extracts or with the OC_{mic} . Considering that the yearly N mineralization represents about 2% of the soil N, absence of a positive correlation with N_{HWE} , constituting 2–5% of the total soil N is surprising. Extraction with hot water has been successfully used to measure plant available N (Broner & Bachler, 1980; Ghani *et al.*, 2003; Curtin *et al.*, 2006) and this fraction is considered to be composed of bioavailable N-containing compounds (Leinweber *et al.*, 1995; Landgraf *et al.*, 2006) but did not seem to contain a significant share of the mineralized N in the studied soil set.

There is, however, a significant positive correlation between both k_{field} and k_{turn} and the absolute as well as relative content of coarse fPOM N, iPOM N and also the sum of POM N (Table 5 and 6). Other studies have indicated that POM may be a primary active sink or source of N (Bird *et al.*, 2002). In a laboratory incubation study, Magid & Kjærgaard (2001) observed a close relationship between changes in POM-N and net-N mineralization. Janzen (1987) reported that LF-N was significantly correlated with net N mineralization in a study involving long-term wheat cropping. In a subsequent study Janzen *et al.* (1992) report

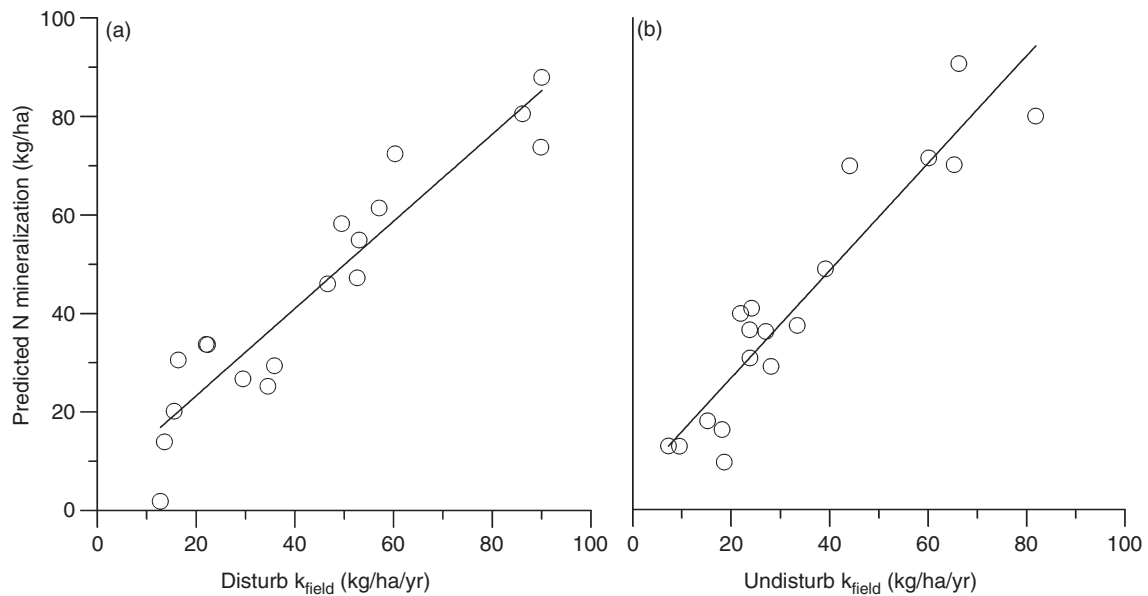


Figure 4 Relation between predicted (kg N/ha/yr) and observed N mineralization (kg/ha/yr) under both disturbed (a) and undisturbed (b) conditions.

that the correlation between LF-N and N mineralization was not as strong nor as consistent as that with soil respiration. The POM C:N ratio is higher than the soil C:N ratio and microbial degradation of POM often results in immobilization of mineralized N (Sollins *et al.*, 1984; Curtin & Wen, 1999). This could explain the generally weak correlations between POM N content and N mineralization. Here, however, a positive correlation between POM C and N mineralization suggests that no immobilization occurred in the incubations. This is mostly linked to the long-term management of these fields. Leaf and root residues from sugarbeets and potatoes, with lower C:N ratios which are known to contribute net N mineralization, are normally ploughed in, while cereal straw residues are normally removed. Moreover, organic fertilizers include mainly slurries with C:N ratios close to six and are applied to these fields.

A significant positive correlation between N mineralization and the content and proportion of iPOM N (expressed as total amount and % of total N) was unexpected since other researchers have found the iPOM fraction to be an intermediary decomposed OM fraction. For example, Sleutel *et al.* (2007) found iPOM to be depleted of lignin dimers, lipids and sterols compared to fPOM in one of the studied soils. Golchin *et al.* (1994) and Köbl & Kögel-Knabner (2004) found *O*-alkyl C (carbohydrates and polysaccharides) to be depleted in occluded compared with free light fractions. N mineralization increased in disturbed soils in 13 out of 18 fields by up to 75%. This is in line with the concept that physical disruption of soils increases microbial accessibility to intrinsically labile but physically protected OM inside aggregates (Six *et al.*, 2000). The increase in N mineralization for our soils was not as large as previously observed by

Ringuet & Bachmeier (2002; 200% increase) and Cabrera & Kissel (1988; 67–343% increase), probably because here soil disturbance was not as drastic as in those studies. For all POM fractions, the correlation was slightly stronger with k_{field} and k_{turn} measured from disturbed than from undisturbed soil. Particularly for iPOM, these data may demonstrate that the disturbance of soil structure could have enhanced the bioavailability of this physically protected N pool.

The C:N ratio of the silt and clay OM fraction, but not its C or N content is significantly and negatively correlated with k_{field} and k_{turn} , which implies that the quality of the silt and clay OM fraction rather than its proportion, determines its contribution to the soil N mineralization. A further subdivision of the silt and clay OM by chemical fractionation revealed a similar trend for the NaOCl-ox, NaOCl-res and the HF-res OM fractions. The absence of a significant positive correlation between the N mineralization and the content or proportion of HF-ext N could be expected since this fraction is assumed to comprise mineral protected N (Mikutta *et al.*, 2006). Other researchers (Chesire *et al.*, 2000; Mikutta *et al.*, 2006) conclude that some amino acids are protected from oxidation by 6% NaOCl (and thus possibly also from mineralization) by occlusion within microaggregates or by interaction with mineral surfaces. However, they were effectively and preferentially removed during mineral dissolution with 10% HF. Mineral binding of organic N has been demonstrated previously. Leinweber & Schulten (2000) found that associations with silicates and pedogenic oxides prevent hydrolysis of a significant part of organic N by 6 M HCl. On the other hand, surprisingly, HF-res N (both absolute and relative) showed a strong positive

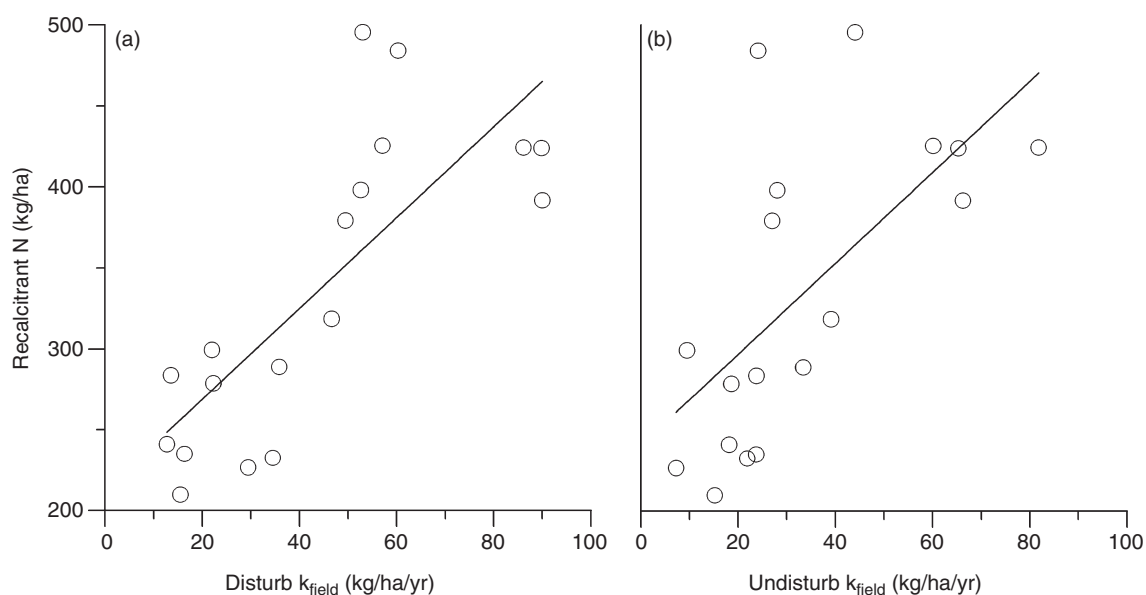


Figure 5 Relation between HF-res N (kg N/ha) and disturbed (a) and undisturbed (b) N mineralization (kg N/ha/yr).

correlation with k_{field} and k_{turn} (Figure 5, Table 6). This fraction is presumed to contain recalcitrant OM, given its resistance to severe chemical treatment but our results strongly suggest that the HF-res OM contains mineralizable N as well. This fraction might contain peptides that are sterically entrapped in larger SOM structures that may will be of old age themselves as previously shown from ^{14}C dating. Sleutel *et al.* (2009) reported an enrichment of peptides and heterocyclic N containing compounds in 6% NaOCl-residues and peptides in 6% NaOCl + 10% HF-residues based on pyrolysis-field ionization mass spectroscopy analysis. These and our findings thus suggest that subsequent 6% NaOCl and 10% HF treatment is not able to isolate all the bioavailable N. A potential explanation for the seemingly contradictory presence of labile N in the residual OM (10% HF treatment residual OM) further may be found in Schulten & Schnitzer (1997). They hypothesized a portion of proteinaceous materials (proteins, polypeptides, peptides and amino acids) in soils trapped in the voids of the three dimensional structure of humic macromolecules. Such humic compounds are probably relatively resistant against mild wet oxidation and would not be extracted by HF. An alternative conceptual model according to Kleber *et al.* (2007), however, envisions different layers of individual OM compounds surrounding low-charge mineral particles (as may mostly be expected in illite dominated clay fractions common in the studied loess belt). The contact zone around mineral particles is enriched in proteinacious material and is surrounded by a hydrophobic zone and an outer kinetic zone of biomolecules that are loosely retained by cation bridging, hydrogen bonding and other interactions. Labile organic N in this kinetic layer may be expected to be mineralizable because of

its high exchange rates with the surrounding soil solution. This theory, however, does not readily explain the presence of labile N in NaOCl treatment + HF treatment residues. Also, while the OM and N in the outer kinetic zone would probably be also oxidizable by triple 6%NaOCl treatment, the NaOCl-oxidizable N did not consistently and positively correlate with the N mineralization.

Another result of interest concerns the strong negative correlations of Al_p and Fe_p contents with N mineralization. Such polyvalent complexed cations would contribute to the general stability of OM macromolecular structures, regardless of whether they occur in humic substances [cf. Schulten & Schnitzer (1997)] or in layered conformations surrounding mineral particles [cf. Kleber *et al.* (2007)]. These data might suggest that the short to medium term silt and clay sized mineralizable N is not withheld by mineral interactions in the studied soils, but may be intermediary stabilized through OM–OM interactions.

Finally, considering the R^2 values of the linear models, substantial variation in N mineralization can be explained by the included independent variables which cover both physical (coarse fPOM N, silt and clay N) as well as chemical fractions (HF-res N and its C:N ratio, NaOCl-ox N). This points out that SOM quality dominantly determines the assessed N mineralization rates, particularly so when incubating soil under 'disturbed' conditions. Heumann *et al.* (2003) also found SOM quality parameters such as a C:N ratio of $<20 \mu\text{m}$ SOM fraction was the best predictor of N mineralization for NW German sandy arable soils. Instead, physical fractions of SOM appear to be the most useful predictors in model 1 which could imply that physical processes exert a larger influence on N mineralization in

undisturbed soil. HF-res OM was a significant predictor in the other three models (model 2, 3 and 4) and this demonstrates the usefulness of combined physical and chemical fractionation for predicting N mineralization. However, assessing the multitude of parameters in models 1, 2 and 4 would be laborious. Omitting less significant predictors such as OC_{mic} for model 1, NaOCl-ox C and $C:N_{HWE}$ for model 2 and $C:N_{HWE}$ for model 4 would only reduce the R^2 values to 0.80, 0.71 and 0.83 for models 1, 2 and 4, respectively. For measuring the parameters of these simplified models, soft ultrasonication and wet sieving at 250 μm to isolate the coarse fPOM is needed followed by wet sieving at 53 μm to isolate the silt and clay sized fraction. Next, chemical treatment of the silt and clay sized SOM three times by 6 h 6% NaOCl and by four times by 2 h 10% HF extraction would be needed to isolate presumably non-mineral bound recalcitrant OM which, however, contains labile N as well.

Conclusion

The SOM fractions obtained from combined physical and chemical fractionation are useful predictors for estimating the annual N mineralization rate under 'disturbed' and 'undisturbed' conditions. Contrary to the findings from several previous studies, coarse fPOM N as well as iPOM N are significant contributors to soil N mineralization. The incorporation of plant litter and slurries with relatively low C:N ratios could explain this result. An unexpected positive correlation between N mineralization and the N content and C:N ratio of NaOCl and HF treatment resistant OM indicates the presence of labile N in this fraction. Such organic N might involve peptides that are sterically entrapped in larger SOM structures that may well be biologically stable themselves. On the other hand mineral bound organic N (i.e. in HF extractable OM) appears to have a very limited contribution. Hence, the methodology was unable to isolate labile from mineral bound and from biochemically stable organic N. However, this approach demonstrates the potential for development of pedotransfer models for N mineralization with measurable N fractions. For practical purposes, the fractionation scheme used in this study needs to and can be simplified to contain no more than three variables, which may be generally relevant for N mineralization. We demonstrate that from such a reduced list of SOM quality variables, models may be developed that are good predictors for N mineralization from a regionally defined soil set. Such a model would have to be calibrated locally and could be useful to incorporate in agronomic decision support systems.

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