Organic matter fractions and N mineralization in vegetable-cropped sandy soils

K. Jegajeevagan[1,2], S. Sleutel[1], N. Ameloot[1], M. A. Kader[3] & S. De Neve[1]

[1]*Department of Soil Management, Ghent University, Coupure Links 653, 9000 Gent, Belgium, [2]Department of Agricultural Chemistry, University of Jaffna, P.O. Box 57, Thirunelveli, Sri Lanka, and [3]Department of Soil Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*

Abstract

Soil organic nitrogen mineralization rates and possible predictors thereof were investigated for vegetable-growing soils in Belgium. Soil organic matter (SOM) was fractionated into sand (> 53 μm) and silt+clay (< 53 μm) fractions. The latter fraction was further separated into 6%NaOCl-oxidation labile (6%NaOCl-ox) and resistant N and C and subsequently into 10%HF-extractable (mineral bound) and resistant (recalcitrant) N and C. The N mineralization turnover rate (% of soil N/year) correlated with several of the investigated N or C fractions and stepwise linear regression confirmed that the 6%NaOCl-ox N was the best predictor. However, the small R^2 (0.42) of the regression model suggests that soil parameters other than the soil fractions isolated here would be required to explain the significant residual variation in N mineralization rate. A next step could be to look for alternative SOM fractionations capable of isolating bioavailable N. However, it would appear that the observed relationships between N fractions and N mineralization may not be causal but indirect. The number of vegetable crops per rotation did not influence N mineralization, but it did influence 6%NaOCl-ox N, probably as an effect of differences in crop residues returned and organic manure supply. However, the nature of this relation between management, SOM quality and N mineralization is not clear. Explanation of correlations between N mineralization and presumed bioavailable N fractions, like the 6%NaOCl-ox N, requires further mechanistic elucidation of the N mineralization process.

Keywords: Nitrogen mineralization, soil organic matter, intensive vegetable production, soil fractionation, sandy soils

Introduction

Realistic estimates of N mineralized from soil organic matter (SOM) are essential for determining the required rate of N fertilizer for cropland production to optimize crop yield and quality while minimizing the adverse impacts of excessive N on the environment. To this end, the identification of useful biological, physical or chemical assays to predict N mineralization from SOM will be crucial.

Laboratory incubations to predict net N mineralization in the field are laborious and overestimations have been usually reported (Verstraete & Voets, 1976; O'Connell & Rance, 1999). Within this context, SOM fractionation might provide a less time-consuming alternative for predicting soil N mineralization.

Different fractionation methods have been applied to obtain indices for SOM mineralization at the level of physically uncomplexed OM (Gregorich & Janzen, 1996), primary particles or particle size fractions (Guggenberger *et al.*, 1995; Balesdent *et al.*, 1998) and organo-mineral complexes or aggregates (Six *et al.*, 2004; Olk & Gregorich, 2006; Kader *et al.*, 2010). However, the majority of these studies were on C mineralization and most researchers focused on physically uncomplexed SOM only. This fraction could be considered as a readily available food or energy source for the soil microbial biomass (Gregorich *et al.*, 2006), but it does not relate well to N mineralization because of its high C/N ratio. Instead, Boone (1994) and Leinweber & Schulten (2002) looked at OM associated with mineral particles of the silt+clay-sized OM. They concluded that the OM associated with heavy

silt+clay-sized fractions should constitute the primary source of potentially available N in various soils. Alternatively, numerous chemical extractions, often by (weak hydrolysing) salt solutions, have been developed to estimate the N-supplying capacity of soils. Ros *et al.* (2010) provided experimental evidence that OM compounds extracted by these methods are often intermediate decomposition waste products in equilibrium with total N. Consequently, extractable N does not equate to biodegradable N. Furthermore, Ros (2011) and Juma & Paul (1984) concluded that extractable OM is indeed not the only source of mineralizable N.

Leinweber & Schulten (2002) confirmed that large amounts of relatively degradable or labile N-containing organic compounds (peptides, amino acids, etc.) are bound to humic macromolecules and to iron and aluminium hydroxides. Extraction by dithionite citrate bicarbonate rendered an additional part of soil N acid hydrolysable. Mikutta *et al.* (2006) extracted soil with HF following NaOCl treatment to subsequently isolate labile and mineral bound C and N. They also found that a major part of soil N was protected from chemical oxidation by mineral association. The ^{14}C ages of isolated fractions were in the order: NaOCl oxidizable OM < bulk soil OM < NaOCl resistant but HF-removable OM < NaOCl and HF-resistant OM. This trend has been confirmed by several authors as well, as reviewed by von Lützow *et al.* (2007). Quantitative data on the relative distribution of N over sand-sized OM, labile OM, mineral bound OM and recalcitrant OM, which clearly differ in both ^{14}C age and governing stabilization mechanisms against microbial degradation, might help in understanding and eventually predicting N mineralization in arable land. To date, however, little experimental data are available that correlate N mineralization to such measurable fractions.

In the present study, we used a sequential fractionation approach involving wet sieving, oxidation by NaOCl and extraction by HF with the explicit aim to separate 'degradable or labile', 'mineral associated and protected' and 'recalcitrant' SOM pools. Via correlation analysis, these N and C fractions were tested as potential predictors of the N mineralization in vegetable-cropped soils in Flanders, Belgium. Their specific history of excessive use of organic fertilizers and incorporation of N-rich crop residues results in generally high N mineralization rates from SOM of about 0.9 up to 1.6 kg N/ha per day (Demyttenaere, 1991). There is, however, a large variation in the N mineralization, which has not been explained to date. We expect a small contribution to N mineralization from HF-resistant N, a large contribution from silt+clay-sized NaOCl-oxidizable N and an intermediate contribution from silt+clay NaOCl-resistant N and sand N. We wanted to test the hypothesis that this distribution over N fractions, rather than soil N content, determines N mineralization for a set of 20 cropland soils. A secondary objective was to test whether and how the number of vegetables in crop rotation influences the distribution of C and N over physicochemical fractions and N mineralization.

Materials and methods

Study area description and farmers' fields

Nineteen different fields, covering three cropping groups, namely i) strictly vegetable production (VE), ii) primarily arable production with inclusion of maximally a single vegetable crop per rotation (AR) and iii) alternating arable-vegetable production (ARVE) were selected in Western Flanders, Belgium (Table 1). The AR rotations primarily included winter wheat, winter barley, sugar beet, field bean, potato and silage maize. Main vegetables grown in the VE group were cauliflower, leek, endive, red and white cabbage, carrot, spinach and Brussels sprout. The annual mean temperature of the study area is 9.7 °C, and the mean annual rainfall is around 800 mm. About 15 kg of soil was collected from the 0 to 20 cm topsoil layer from each field in September 2008 with an auger. The sampling pattern was designed to cover the entire field in a zigzag pattern, and samples were mixed into a bulk soil sample. Three representative undisturbed field bulk density measurements were taken from the middle of the topsoil layer (10–15 cm).

Nitrogen mineralization

A 14-week incubation experiment was set up to estimate N mineralization from native SOM. Thoroughly mixed air-dried soil (65 g of dry soil) was filled in PVC tubes with an inner diameter of 0.033 m and 0.054 m of length. The soil was brought to a bulk density of 1.4 Mg/m^3 by compaction until a predetermined volume was achieved. The moisture content of the soil was then adjusted to a level of 50% water filled pore space by the addition of deionized water. The tubes were covered with a layer of gas-permeable parafilm, the total weight of the tubes was recorded and the tubes were incubated at a temperature of 20 °C for 14 weeks. We prepared 21 tubes per field (7 sampling events × 3 replicates), resulting in a total number of 399 tubes. The moisture content was monitored regularly during incubation by weighing the tubes and was kept constant by adding deionized water as required. Samples were taken randomly and destructively after 2, 4, 6, 8, 10, 12 and 14 weeks by removing the soil from the intact tubes. The soil was mixed thoroughly and 10 g moist soil was analysed for its mineral N (NO_3^--N and NH_4^+-N) content by extraction with a 1M KCl solution (1:5 soil weight (g)/extractant volume (mL)). The mineral N concentration in the extract was measured colorimetrically with a 'continuous flow auto-analyzer' (Chemlab System 4, Skalar, the Netherlands). N

Table 1 Basic soil properties of the sampled sandy cropland soils under arable (AR), vegetable (VE) or alternating arable-vegetable (ARVE) production

Location	Soil	pH$_{KCl}$ (–)	Total C (g/kg)	Total N (g/kg)	C/N (–)	Sand (%)	Silt (%)	Clay (%)
Staden	ARVE1	6.71	9.39	0.93	10.1	53	40	7
Poelkapelle	ARVE2	7.06	11.7	1.06	11.1	54	36	10
Nevele	ARVE3	5.00	11.5	0.92	12.5	43	47	10
Aalter	ARVE4	7.16	10.4	0.97	10.8	34	58	8
Lendelede 1	AR1	5.45	7.3	0.63	11.6	49	44	7
Lendelede 2	AR2	5.20	6.9	0.55	12.6	51	42	7
Otegem 1	AR3	5.47	9.7	0.97	9.9	27	63	10
Otegem 2	AR4	6.25	7.8	0.70	11.2	25	64	11
Otegem 3	AR5	5.29	16.4	1.62	10.2	23	66	11
Otegem 4	AR6	4.91	10.0	0.99	10.1	20	68	12
Deurle	VE1	5.14	17.5	1.24	14.1	79	17	4
Kruishoutem	VE2	5.32	10.9	0.81	13.6	78	15	7
Aarsele	VE3	6.07	11.5	0.97	11.8	49	45	6
Vosselare	VE4	5.91	15.8	1.14	13.8	46	44	10
Torhout	VE5	5.86	14.9	1.20	12.4	79	17	4
Handzame	VE6	5.79	10.2	0.96	10.6	69	26	5
Reningelst	VE7	7.21	11.3	1.15	9.8	35	54	11
Heule	VE8	6.03	6.4	0.55	11.7	51	40	9
Waregem	VE9	4.71	7.95	0.69	11.5	83	13	4
Crop rotation								
ARVE		6.4 ± 1.0a[b]	10.8 ± 1.0	0.97 ± 0.0	11.1 ± 1.0	46 ± 9ab	45 ± 10ab	9 ± 1ab
AR		5.4 ± 0.5b	9.7 ± 3.5	0.91 ± 0.4	10.7 ± 0.7	36 ± 14a	58 ± 12a	10 ± 2a
VE		5.8 ± 0.8ab	11.8 ± 3.6	0.97 ± 0.23	12.1 ± 1.5	63 ± 18b	30 ± 16b	7 ± 3b
ANOVA[a]		*	N.S.	N.S.	N.S.	*	*	*

[a]*significantly different at the 5% significance level based on ANOVA F-test; N.S. not significantly different based on ANOVA F-test. [b]a,b Means followed by a different letter are significantly different at 5% significance level according to Duncan's multiple range test.

mineralization rates were estimated by fitting a zero-order model:

$$N(t) = N_0 + k.t \qquad (1)$$

where t is the time (in weeks), $N(t)$ is the amount of mineral N in the soil at time t (mg N/kg), N_0 is the initial amount of mineral N (mg N/kg) and k is the mineralization rate (mg N/kg per week). A temperature correction function (De Neve & Hofman, 1996) was used to recalculate the N mineralization rate obtained in the laboratory at 20 °C to an N mineralization per year under field conditions (kg N/ha per year) at a yearly average temperature of 9.7 °C. The ratio of k at 20 °C and at 9.7 °C was 2.40 and analogously a 1-year mineralization at 9.7 °C corresponds to 152 days incubation under the specific laboratory conditions. The yearly N mineralization was calculated by extrapolation to 152 days, assuming a linear course of the N mineralization past the duration of the laboratory incubation experiment. A relative yearly soil N 'turnover rate' (% of soil N/year) was calculated by dividing the calculated yearly N mineralization (kg N/ha per year) by the total N content in the topsoil layer (kg N/ha).

Microbial biomass C

Soil microbial biomass C (OC_{mic}) was determined by the fumigation-extraction method (Vance *et al.* 1987) at the end of the 4th and 14th weeks of incubation. The K$_2$SO$_4$ extracts before and after fumigation with CHCl$_3$ were filtered through paper filters (Whatman 5) and were analysed for dissolved OC with a Total OC analyzer (TOC-V$_{CPN}$; Shimadzu corp., Kyoto, Japan). To correct for the incomplete release and extraction of soil microbial biomass C, an extraction efficiency value of 0.45 according to Joergensen (1996) was used. The moisture content from each soil sample was determined from 10 g of soil.

Physicochemical fractionation of soil organic matter

An ultrasonication-wet sieving method (Schulten & Leinweber, 1995; Amelung *et al.*, 1998; Schmidt, 1998) was used to fractionate SOM into sand size (> 53 μm) (sand C and N) and silt+clay size (< 53 μm) (silt+clay C and N) fractions. To breakdown macro and micro aggregates, samples were dispersed by ultrasonication (20 g soil in

100 mL deionized water) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26). The ultrasonic probe was first calibrated by heating 150 mL deionized water in a Dewar vessel for 2 min. A low energy input of 60 J/mL was used to protect particulate organic matter (POM) from disruption, while achieving dispersion of sand-sized aggregates (Amelung & Zech, 1999).

After ultrasonic dispersion, the > 53 μm fraction was isolated by wet sieving the dispersed soil slurry through a 53 μm mesh sieve. The POM retained on the sieve together with sand was collected in preweighed aluminium cups. The water and soil fraction passing through the 53 μm sieve, the silt+clay size fraction, were also collected in preweighed aluminium cups. All fractions were oven-dried at 105 °C, left to cool down in desiccators and weighed and stored in glass vials.

The silt+clay size fraction 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 by 6% NaOCl and mineral extraction by 10% HF-acid. The procedure results in the isolation of a chemically stable 6% NaOCl-resistant SOM fraction composed of mineral protected as well as biochemically recalcitrant OM, and a biochemically nonbound recalcitrant SOM fraction resistant to 10% HF treatment (Figure 1). A 5 g sample of the isolated silt+clay size fraction 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 in between oxidation cycles and were ultimately washed one time with 1M NaCl and three times with deionized H$_2$O. After drying and weighing, a subsample was collected for total C and N analysis. Then, 3 g of the oxidation residue was treated four times with 20 mL 10% HF to dissolve and remove mineral constituents and mineral-bound OM. Extraction residues were washed five times with deionized water to remove salts and residual HF and were decanted into preweighed aluminium cups, dried and weighed.

Carbon and nitrogen analysis

The total C and N content of a 200–800 mg subsample of the isolated soil fractions was analysed with a Variomax CNS-analyzer (Elementar Analysensysteme, Germany). Because all sampled soils were free from carbonates, the measured total C content equals the soil organic C (SOC) content. Measurement of N in the HF-treated samples was impossible by elemental analysis due to the presence of residual Ca or Si fluorides, which caused F volatilization that interfered with the N measurements. For these samples, the total N was determined through a modified Kjeldahl method. Absolute amounts of C and N in the different
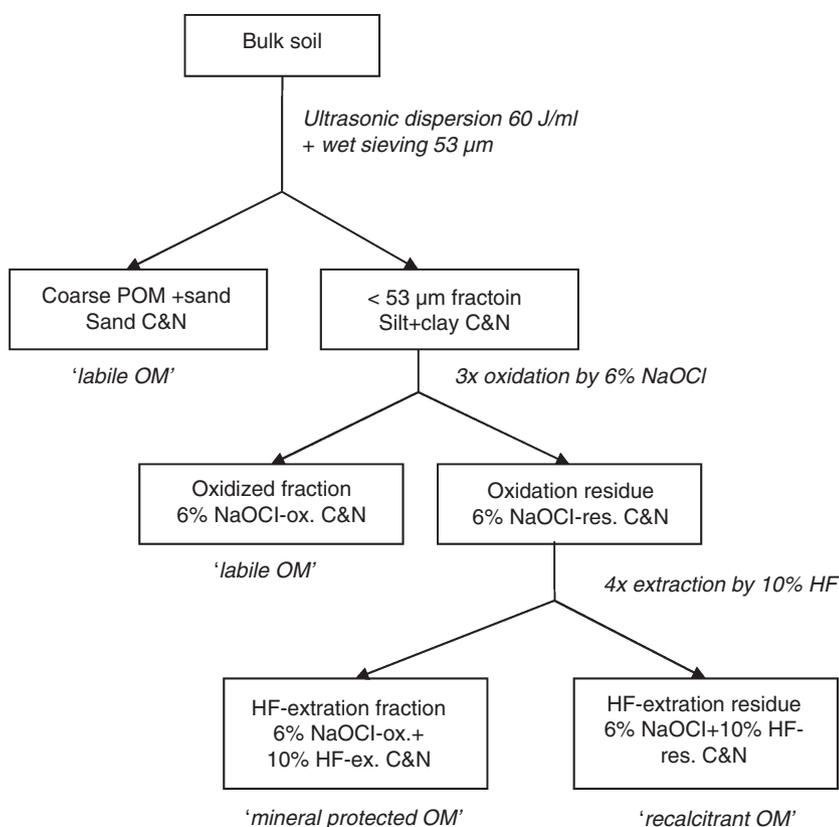
Figure 1 Combined physical and chemical fractionation scheme to isolate presumed labile, mineral protected and recalcitrant organic matter.

fractions, expressed in g/kg, were calculated from the dry matter weight of each fraction and its percentage of C and N. The amount of 10% HF-extracted C and N was quantified by subtraction of C and N in the 6% NaOCl residues and the 6% NaOCl+10% HF-treatment residues.

Statistical analysis

All statistical analyses were carried out with the SPSS 15.0 package (SPSS Inc., USA). ANOVA and Duncan's *post hoc* mean comparison tests were conducted to detect significant differences between means of the cropping groups for the relative and absolute C and N contents of the physicochemical fractions and for the N mineralization rates. Correlation analysis was used to investigate the relation between the N and C in isolated physicochemical fractions and the annual N mineralization. Absolute (kg N kg/ha per year) and relative (% of total N mineralized/year) annual N mineralization rates were correlated to the absolute N and C contents (in g/kg) and the relative N and C proportions (in % of soil N and SOC), respectively. Multivariate linear regression was conducted with the N mineralization rates as dependents and the relative proportions of SOC and N of all the isolated fractions (in % of soil N or SOC) along with their C/N ratios, microbial C and general soil properties as independents using SPSS's 'stepwise linear regression' function. The successive selection of independents 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-in = 0.05) or removed (if F > P-out = 0.10).

Results

The set of soils represented a wide range in N and C contents (0.55–1.62 g N/kg and 6.41–17.47 g OC/kg) with an average C/N ratio of 11.5 ± 1.3 (Table 1). During incubation, the amount of mineral N increased linearly with time in all soils, showing considerable variation between the replicates. The average recalculated annual field N mineralization was 164 ± 28.1 kg N/ha per year (for the 0–30 cm layer at an average field temperature of 9.7 °C and at bulk density 1.4 Mg/m^3). The mean mineralization rate (Table 2) did not significantly differ among the cropping groups. The relative yearly N mineralization did not significantly differ between the cropping groups and was negatively correlated with the soil N and C content ($P < 0.01$).

OC and N in physicochemical fractions and Microbial biomass C

Two different N fractions were isolated by the ultrasonication-wet sieving procedure, namely N in sand

Table 2 N mineralization rates determined at 20 °C in laboratory incubations (means ± SE) and annual absolute and relative N mineralization recalculated for field conditions (9.7 °C mean annual temperature, 30 cm layer)

Soil	N Mineralization rate (20 °C) (mg N/kg per week)	N Mineralization Field (kg N/ha per year)	Relative field N mineralization (% of soil N/year)
ARVE1	1.92 ± 0.25	175 ± 23	4.5 ± 0.6
ARVE 2	2.35 ± 0.46	214 ± 45	4.8 ± 1.0
ARVE 3	1.87 ± 0.44	171 ± 40	4.4 ± 1.0
ARVE 4	1.72 ± 0.27	157 ± 25	3.9 ± 0.6
AR1	1.78 ± 0.31	162 ± 28	6.1 ± 1.1
AR 2	1.36 ± 0.43	124 ± 39	5.4 ± 1.7
AR 3	2.11 ± 0.38	192 ± 35	4.7 ± 0.9
AR 4	1.32 ± 0.15	120 ± 14	4.1 ± 0.5
AR 5	2.09 ± 0.69	191 ± 63	2.8 ± 0.9
AR 6	1.68 ± 0.22	153 ± 20	3.7 ± 0.5
VE1	1.84 ± 0.28	168 ± 26	3.2 ± 0.5
VE2	1.56 ± 0.25	142 ± 23	4.2 ± 0.7
VE3	2.04 ± 0.32	186 ± 29	4.6 ± 0.7
VE4	1.78 ± 0.31	162 ± 28	3.4 ± 0.6
VE5	1.45 ± 0.27	132 ± 25	2.6 ± 0.5
VE6	2.12 ± 0.40	193 ± 36	4.8 ± 0.9
VE7	2.17 ± 0.26	198 ± 24	4.1 ± 0.5
VE8	1.27 ± 0.19	116 ± 17	5.0 ± 0.8
VE9	1.78 ± 0.20	162 ± 18	5.6 ± 0.6
Crop rotation			
ARVE	1.97 ± 0.27	179 ± 25	4.4 ± 0.4
AR	1.72 ± 0.34	157 ± 31	4.5 ± 1.2
VE	1.78 ± 0.31	162 ± 28	4.2 ± 1.0
ANOVA[a]	N.S.	N.S.	N.S.

ARVE, Alternating arable-vegetable; VE, vegetable; AR, arable.
[a]N.S. not significantly different at the 5% significance level based on ANOVA F-test.

and silt+clay size fractions. The average amounts of sand N and C or silt+clay N and C were not significantly different among the cropping groups (Table 3). The relative proportion of silt+clay N of the VE group was significantly different from the arable-cropping groups (Table 3). The C/N ratios of the whole soil samples ranged between 9–14, and the C/N ratio of the sand fraction (19.6 ± 7.3) was larger than of the silt+clay fraction (9.1 ± 1.6). The C/N ratio of the silt+clay fraction in the VE group was significantly ($P < 0.05$) greater than that for the other cropping groups.

The 6% NaOCl treatment removed 0.28–1.18 g N/kg from the silt+clay fraction, and a significantly smaller proportion of 6% NaOCl-ox N was found in the VE (54% of soil N) compared to the ARVE-cropping group (68% of soil N). The 6% NaOCl residues were subsequently treated by 10% HF. The N and C released by HF is presumed to represent

Table 3 Mean (±SE) N and C content of physicochemical fractions of sandy cropland soils under arable (AR), vegetable (VE) and alternating arable-vegetable (ARVE) production

	Sand size fraction (> 53 μm)			Silt+Clay size fraction (< 53 μm)								
				6% NaOCl oxidizable			6% NaOCl resistant					
							10% HF extractable[a]			10% HF resistant		
Crop rotation	N (g/kg)	C (g/kg)	C/N (−)	N (g/kg)	C (g/kg)	C/N (−)	N (g/kg)	C (g/kg)	C/N (−)	N (g/kg)	C (g/kg)	C/N (−)
ARVE	0.09 ± 0.01a	1.9 ± 0.2	21.1 ± 4.2	0.66 ± 0.09a[c]	4.75 ± 0.81	7.2 ± 0.4	0.15 ± 0.02ab	0.54 ± 0.30a	3.7 ± 2.2	0.07 ± 0.03	1.60 ± 0.11	25.5 ± 8.8b
AR	0.08 ± 0.02a	1.6 ± 0.4	21.1 ± 6.4	0.61 ± 0.32ab	4.61 ± 1.8	8.0 ± 1.5	0.12 ± 0.06a	0.59 ± 0.40a	6.4 ± 5.0	0.09 ± 0.05	1.48 ± 0.57	16.8 ± 4.3a
VE	0.13 ± 0.03b	2.1 ± 1.1	16.2 ± 9.8	0.53 ± 0.17b	4.4 ± 2.0	8.0 ± 2.1	0.19 ± 0.06b	1.50 ± 0.67b	8.2 ± 3.5	0.10 ± 0.07	2.52 ± 1.39	26.2 ± 5.5b
ANOVA[b]	*	N.S.	N.S.	*	N.S.	N.S.	*	*	N.S.	N.S.	N.S.	*

[a]calculated as the difference between 6% NaOCl C and N and the 10% HF-resistant C and N. [b]*significantly different at the 5% significance level based on ANOVA F-test; N.S. not significantly different; for 10%HF-extractable C, 10% HF resistant C and the sand fraction C Brown–Forsythe's statistic with the Games–Howell *post hoc* test was used instead because the condition of homoscedasticity was not fulfilled. [c]a,b Means followed by a different letter are significantly different at 5% significance level according to Duncan's multiple range test.

silt+clay mineral bound N (6% NaOCl-res+10% HF-ex N and C), whereas N and C not released by HF represents chemically resistant or recalcitrant N (6% NaOCl+10% HF-res N and C) (Mikutta *et al.*, 2006). The amount of N released by 10% HF extraction varied between 0.01 and 0.27 g N/kg. The 6% NaOCl+10% HF-res N varied between 0.02 and 0.21 g N/kg (Table 3). The 10% HF-treatment strongly and selectively removed N over C, resulting in a much larger C/N ratio of the 6% NaOCl+10% HF-res fraction than of the 6% NaOCl-ox or silt+clay fractions. The 6% NaOCl-res+10% HF-ex N was significantly higher ($P < 0.05$) for the VE (0.19 ± 0.06 g N/kg) than for the AR (0.15 ± 0.02 g N/kg)-cropping group. This difference was also significant for C between the VE- and ARVE-cropping groups. The amount of 6% NaOCl+10% HF-res N and C was not significantly different between any of the cropping groups. The C/N ratio of the 6% NaOCl+10% HF-res fraction was significantly greater ($P < 0.05$) for the AR-cropping group than for the VE- and ARVE-cropping groups.

The proportion of OC_{mic} relative to SOC was on average $0.82\% \pm 0.46\%$ of SOC, which is not particularly large when compared to 2–5% OC_{mic} relative to SOC reported by Anderson & Domsch (1989). No significant correlation was found between the N mineralization rate and OC_{mic} (data not show).

Correlations between N mineralization and physicochemical fractions

Pearson's correlation coefficients between the absolute annual N mineralization rate or the relative yearly N mineralization and the C and N in soil fractions on an absolute basis (g/kg) or on a relative basis (% of soil N,% of SOC), and the C/N ratios are listed in Table 4. The N mineralization rate was positively correlated to the content of silt+clay N, the content of 6% NaOCl-ox N and C and the proportions of silt+clay N and 6% NaOCl-ox N ($P < 0.05$). Negative correlations were found with the proportions of 6% NaOCl-res C, sand N and 6% NaOCl-res+10% HF-ex C and the C/N ratios of the silt+clay and 6% NaOCl-res+10% HF fractions.

A stepwise linear regression was conducted between the yearly N mineralization (kg N/ha per year) and the absolute contents and relative proportions of C and N of the isolated fractions along with their C/N ratios and resulted in following model: yearly N mineralization = 114 + 85.0 6% NaOCl-ox N ($R^2 = 0.417$; $P < 0.01$, $N = 19$). None of the measured soil characteristics significantly explained the residual variation in net N mineralization not accounted for by the 6% NaOCl-ox N. However, the variance inflation factors of the excluded variables silt+clay N, NaOCl-ox C and soil N were high (> 3), and all were strongly correlated to the 6% NaOCl-ox N ($r = 0.95$, 0.86 and 0.91,

Table 4 Pearson's correlation coefficients between the N mineralization rate, expressed as kg N/ha per year and the soil C and N content, C and N contents or relative proportions of soil C and N of Soil organic matter (SOM) fractions and their C/N ratios, microbial biomass C after 4 and 14 weeks of incubation and the soil sand, silt and clay percentages. (Only variables which significantly correlated with the N mineralization are shown, next to soil N and C content)

	Correlation coefficient with N mineralization (in kg N/ha per year)
Soil N	0.594**
Soil C	0.350
Silt+clay N	0.577**
6%NaOCl-ox N	0.646**
6%NaOCl-ox C	0.501*
Silt+clay OM C/N	−0.487*
6%NaOCl-res+10% HF-ex OM C/N	−0.506*
Proportion of sand N	−0.534*
Proportion of silt+clay N	0.561*
Proportion of 6%NaOCl-ox N	0.509*
Proportion of 6%NaOCl-res C	−0.510*
Proportion of 6% NaOCl-res+10%HF-ex C	−0.535*

*Correlation is significant at $P < 0.05$. **Correlation is significant at $P < 0.01$.

respectively; $P < 0.01$). This demonstrates multicollinearity and redundancy between these predictor variables.

Discussion

Over the entire set of soils, the N mineralization rate varied substantially and it was correlated with soil N or SOC (Table 4). This corresponds with other studies, where such relationships have frequently been found and field N mineralization has then been estimated (e.g. Camargo *et al.*, 2004; Sharifi *et al.*, 2007; Benintende *et al.*, 2008). The recalculated annual field N mineralization rate and relative yearly N mineralization did not significantly differ between the cropping groups (Table 2). Mean contents of SOC and soil N and bulk soil C/N ratios of the three cropping groups were also statistically similar. Crop rotation was thus not found to control soil N content or N mineralization. The VE group had a larger proportion of sand and less silt than the ARVE and AR groups and manifested through different moisture retention characteristics this could have affected N mineralization. However, such an effect was most probably excluded from the laboratory incubations by establishment of a fixed water-filled pore space level. Moreover, there was only minor variation in the clay fraction between the crop rotation groups, and so the potential of these soils for

mineral binding of N was probably similar. The small correlation coefficient between the N mineralization rate and soil N content suggests that other factors as well governed the N mineralization process, only a specific fraction of the organic N was mineralized, or both possibilities were operative. The yearly N turnover rate was negatively related to soil N and soil C. One possible explanation is that storage of soil N in these croplands with differing management is mainly accomplished by build-up of less bioavailable N. To further investigate the relation between SOM quality and N mineralization capacity, we used physicochemical fractionation to quantify SOM quality.

The fractionation data revealed important differences in SOM quality over the studied soils and more specifically in the proportion of labile N pools. First, the proportion of sand fraction N was negatively correlated to the N mineralization rate, suggesting that there was but a limited net mineral N release or even immobilization upon sand OM decomposition, regardless of crop rotation. The sand N contents were larger in the VE soils than in the other soils (Table 3). This is probably a resultant of commonly larger inputs of organic matter from fresh crop residues and animal manures compared to the ARVE and AR soils. Generally, most N mineralization may be expected to derive from the silt+clay fraction (Parfitt & Salt, 2001), which constituted on average 87% of the soil N. The content of silt+clay N was positively correlated with the N mineralization rate but with a slightly smaller correlation coefficient r than soil N content. The silt+clay N fraction is far from a single uniform kinetic soil N pool. Indeed, both labile N-containing compounds like peptides as well as recalcitrant N-heterocycles have been found to be enriched with decreasing particle size (Schulten & Leinweber, 1995). In addition, a substantial share of intrinsically easily degradable proteinaceous N is partly protected through binding to minerals or other SOM components, as evidenced by Leinweber & Schulten (2002) and Ding & Henrichs (2002) among others. Thus, both biochemistry and strength of binding to the soil mineral phase or SOM result in a continuum of degradability of silt+clay fraction N. The chemical fractionation used in this study attempted to separate this continuum into meaningful N pools, that is, with differing degree of stability against microbial degradation.

Figure 2 indicates that the major part of silt+clay N (65–75% of silt+clay N) was oxidized by 6% NaOCl. A selective removal of N over C occurred in all soils but may not be universal. For example, Sleutel *et al.* (2009) showed that 6% NaOCl primarily removed long-chained lipids, sterols and lignin dimers from cultivated (postpodzol) sandy cropland soils. The proportion of 6% NaOCl-res N was significantly greater in the VE-cropping group. Hence, apparently, more chemically stable N was found in the VE soils, which have received frequent manure N inputs, continuous N-rich crop
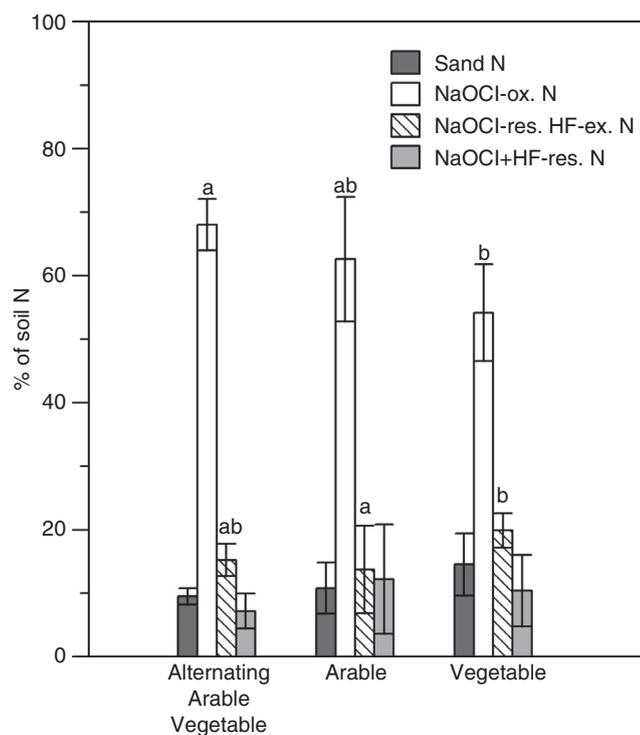
Figure 2 Average relative distribution (% of soil N) of sand N (presumed labile N), 6%NaOCl-oxidizable (presumed labile N), 6% NaOCl-resistant but 10%HF-extractable N (presumed mineral protected) and 6%NaOCl and 10%HF-resistant N (presumed recalcitrant N) of cropping groups.

residues and large mineral fertilizer N inputs over the past 3 –4 decades.

The significant ($P < 0.05$) positive correlation between the content of 6% NaOCl-ox N and the yearly N mineralization ($r = 0.646$) would suggest that this chemically labile N fraction coincides with a relatively more biodegradable or mineralizable soil N pool compared to silt+clay N and soil N. On the basis of ^{14}C dating, Zimmermann *et al.* (2007) and Jagadamma *et al.* (2010) found the 6% NaOCl residue to contain an older C fraction. In contrast, based on regression analysis, Mikutta & Kaiser (2011) and Sleutel *et al.* (2010) found oxidizability by 6%NaOCl to be a poor measure for labile or bioavailable C. However, these studies all looked at C and not N mineralization and relative contributions of SOM protection mechanisms may be very different for C and N. While C is present in a spectrum of biomolecules ranging from labile (carbohydrates and lipids) to more stable (lignin components, suberin, waxes), most N is part of proteins and amino sugars, which are considered less intrinsically stable biomolecules (Kögel-Knabner, 2002). Proteinaceous materials are estimated to comprise minimally 40% (Schulten & Schnitzer, 1998) and maximally 80% of soil N (Knicker, 2000), with a smaller share of heterocyclic N. In addition, distributions of N and C over physical

fractions differ, with a general decreasing C/N ratio with smaller particle size and stronger affinity of soil N to mineral binding. Consequently, oxidizability or extractability of soil N and C may not necessarily match. In line with the present study, Kader *et al.* (2010) found a positive correlation between the yearly N mineralization (kg N/ha) in silty arable soils and 6% NaOCl-ox N. However, they also found a positive correlation with the 6% NaOCl-res N, so the result was ambiguous.

An attempt was then made to further meaningfully divide the on average 27% of soil N left in the 6% NaOCl residues into either mineral bound N or in inherently recalcitrant N via subsequent 10% HF removal of clay minerals and bound OM. Most of the 6% NaOCl-res N was removed during mineral dissolution with 10% HF, suggesting that a substantial part of it was mineral bound. Other researchers (Chesire *et al.*, 2000; Mikutta *et al.*, 2006) also concluded that some amino acids are protected from oxidation by 6% NaOCl by occlusion within microaggregates or by interaction with mineral surfaces. This 6% NaOCl-res+10% HF-ex N was significantly larger in the VE than in the AR group, which however does not match with the smaller content of silt and clay of the VE group (Table 1). This contradiction raises questions about the selectivity of the chosen sequential 6% NaOCl and 10% HF treatment to extract 'mineral protected' OM. Nonetheless, the data show that cropping system did appear to affect this stable presumed N fraction. The differences in 6% NaOCl+10% HF-res N between the cropping groups on the other hand were very small and insignificant, which seems logical as this fraction is presumed to coincide with a recalcitrant OM pool. Sleutel *et al.* (2010) and Kader *et al.* (2010), however, found that 6% NaOCl+10% HF-res C and N correlated to C and N mineralization in arable soils. They therefore concluded that sequential 6% NaOCl and 10% HF extraction failed to isolate a truly biochemically inert SOM fraction. Furthermore, an increasing number of studies consider biochemical recalcitrance as a relatively insignificant stabilization mechanism of SOM (e.g. Kleber & Johnson, 2010; Schmidt *et al.*, 2011). The unresponsiveness of the 6% NaOCl+10% HF-res N and C to crop rotation and the lack of correlations with N mineralization in the present study would, nonetheless, suggest that this fraction is biologically stable. Yet, conflicting data between this and previous studies employing HF extraction demonstrate that it cannot separate kinetically different SOM pools universally.

In synthesis, the control of SOM quality on N mineralization appeared to exceed that of SOM quantity, soil texture or pH as only 6%NaOCl-ox N was withheld as an explanatory variable of yearly N mineralization in a stepwise linear regression analysis. However, considering the small R^2 value of the stepwise linear model, substantial residual variation in N mineralization was not explained. Consequently, SOM quality does not appear to be the sole

dominant factor controlling the N mineralization rate. This acknowledges the argumentation by Ros (2012) that any extractable OM fraction could not be expected to account for all the complex interactions between biological, chemical, and physical factors that control N mineralization. Other studies on sandy arable soils in north-west Europe (e.g. Heumann *et al.*, 2003; Springob & Kirchmann, 2003; Sleutel *et al.*, 2011) have pointed to a lasting influence of land-use history on current day SOM quality and stability. From a meta-analysis of literature, Ros (2012) concluded that positive relations between soil N supply and most soil N fractions are probably indirect via mutual correlations with soil N content. In line, for the investigated set of sandy cropland soils N mineralization correlated to soil N content, 6% NaOCl-ox N and silt+clay N and all were mutually correlated as well. Their large variance inflation factors, calculated in the stepwise linear regression analysis, revealed redundancy among these variables. This suggests that soil N, silt+clay N and 6% NaOCl-ox N explain a very similar part of variation in the N mineralization rate. Fractionation by subsequent removal of sand fraction OM and chemically labile OM therefore does, at least for this set of soils, not seem to isolate soil N fractions with a biodegradability that is substantially different from bulk SOM. Addressing our second objective, sandy vegetable-cropped soils with differing number of vegetables in rotation were not found to have a different N turnover.

Measures of biological, chemical and physical factors controlling SOM decomposition other than the ones yielded by our fractionation approach may better predict N mineralization. Given the complexity of the interactions between all these factors, such soil parameters may well be specific to combinations of climate, soil type and management. For example, Heumann *et al.* (2011) investigated the molecular-chemical composition of SOM of sandy croplands in north-west Germany and found that plant sterols were the SOM components to correlate best with N mineralization. Such detailed molecular-level investigations, however, may not be repeatable for large numbers of fields, limiting their usefulness as inputs to predictive models. In addition, a quantification of alternative particulate OM fractions may be valuable as well, such as silt-sized free OM. Alternatively, clay-N might also be a better predictor than silt+clay N, as the clay fraction is generally enriched in N in microbial biomass and accumulated metabolites (Schulten & Leinweber, 1995). However, physical separation of the clay fraction is very laborious. Lastly, isolation of the fraction with the largest predictive power, namely the 6% NaOCl-ox N, may perhaps be further optimized to better match short- to medium-term mineralizable N, as 6% NaOCl treatment removed about 2/3th of the soil N, by far more than the yearly mineralization of 2–4% of soil N. A less aggressive agent (e.g. 2–4% NaOCl) might be a better proxy for mineralizable soil N.

However, there is no solid mechanistic basis that links the chemical oxidizability of OM to its biodegradability. The data indicate that the relationship between NaOCl-ox N and N mineralization is indirect via a mutual relation with soil N, as predicted by Ros (2012), and if so, lowering the concentration of NaOCl would not matter. Analogously, this leads to the conclusion that oxidation by NaOCl failed to separate a subfraction of the silt+clay OM with distinct biodegradability. Alternatively, separation of (hot) water-extractable OM, directly following wet sieving at 53 μm, could have reduced the composite nature of the silt+clay and 6% NaOCl-ox OM fractions. Such a step possibly would have enabled separate quantification of the most labile part of the silt+clay OM and 6% NaOCl-ox OM, as water-extractable OM has often been shown to coincide with readily biodegradable OM (e.g. Gregorich *et al.*, 2003).

Conclusion

From this study on sandy croplands of north-west Europe primarily used for vegetable production, we found that the annual N mineralization was relatively less with increasing N content, possibly resulting from a smaller biodegradability of the SOM. However, a strong collinearity between soil N and N in several of the isolated physicochemical fractions suggests these variables explain a similar part of the variation in the N mineralization rate. We could therefore not conclude that SOM quality exerts a stronger control on N mineralization than soil N or C content, while it was apparent that the control by soil texture or pH was limited in these soils. Specifically, the content of NaOCl-ox N best explained N mineralization. Nonetheless, oxidation by 6% NaOCl does not appear to mimic biological degradation of organic N, as previously postulated, and a large discrepancy exists between the oxidizable soil N (60–80% of soil N) and the yearly mineralizable N (2–4% of soil N). The isolated presumed 'mineral associated' and 'recalcitrant' SOM fractions could be more biologically stable as their N content did not correlate to soil N mineralization. There was a large residual variation in N mineralization, unexplained by soil N content or any of the physicochemical OM fractions. For one, the number of vegetable crops in rotation significantly affected the chemical liability of soil N, but it did not affect the yearly N mineralization. This calls for alternative approaches to soil fractionation to identify and quantify the biological, chemical and physical factors that control N mineralization.

References

Amelung, W. & Zech, W. 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. *Geoderma*, **92**, 73–85.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E. & Flach, K.W. 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. *Soil Science Society of America Journal*, **62**, 172–181.

Anderson, T.H. & Domsch, K.H. 1989. Ratios of microbial biomass carbon to total organic-carbon in arable soils. *Soil Biology and Biochemistry*, **21**, 471–479.

Balesdent, J., Besnard, E., Arrouays, D. & Chenu, C. 1998. The dynamics of carbon in particle-size fractions of soil in a forest-cultivation sequence. *Plant and Soil*, **201**, 49–57.

Benintende, M.S., Benintende, M.C., Sterren, M.A. & De Battista, J.J. 2008. Soil microbiological indicators of soil quality in four rice rotations systems. *Ecological Indicators*, **8**, 704–708.

Boone, R.D. 1994. Light-fraction soil organic matter - origin and contribution to net nitrogen mineralization. *Soil Biology and Biochemistry*, **26**, 1459–1468.

Camargo, F.A.O., Gianello, C. & Tedesco, M.J. 2004. Soil nitrogen availability evaluated by kinetic mineralization parameters. *Communications in Soil Science and Plant Analysis*, **35**, 1293–1307.

Chesire, M.V., Dumat, C., Fraser, A.R., Hillier, S. & Staunton, S. 2000. The interaction between soil organic matter and soil clay minerals by selective removal and controlled addition of organic matter. *European Journal of Soil Science*, **51**, 497–509.

De Neve, S. & Hofman, G. 1996. Modeling N mineralization of vegetable crop residues during laboratory incubations. *Soil Biology and Biochemistry*, **28**, 1451–1457.

Demyttenaere, P. 1991. *N dynamics in the soils of the West-Flanders vegetable region*. PhD thesis, Ghent University, Ghent.

Ding, X. & Henrichs, S. 2002. Adsorption and desorption of proteins and polyamino acids by clay minerals and marine sediments. *Marine Chemistry*, **77**, 225–237.

Gregorich, E.G. & Janzen, H.H. 1996. Storage of soil carbon in the light fraction and macroorganic matter. In: *Structure and organic matter storage in agricultural soils* (eds M.R. Carter & B.A. Stewart), pp. 167–190. Lewis Publishers CRC Press, Boca Raton.

Gregorich, E.G., Beare, M.H., Stoklas, U. & St-Georges, P. 2003. Biodegradability of soluble organic matter in maize-cropped soils. *Geoderma*, **113**, 237–252.

Gregorich, E.G., Beare, M.H. & McKim, U.F. 2006. Chemical and biological characteristics of physically uncomplexed organic matter. *Soil Science Society of America Journal*, **70**, 975–985.

Guggenberger, G., Zech, W., Haumaier, L. & Christensen, B.T. 1995. Land use effects on the composition of organic matter in particle size separates of soil. II. CPMAS and solution 13C NMR analysis. *European Journal of Soil Science*, **46**, 147–158.

Heumann, S., Bottcher, J. & Springob, G. 2003. Pedotransfer functions for the pool size of slowly mineralizable organic n in sandy arable soils. *Journal of Plant Nutrition and Soil Science*, **166**, 308–318.

Heumann, S., Schlichting, A., Bottcher, J. & Leinweber, P. 2011. Sterols in soil organic matter in relation to nitrogen mineralization in sandy arable soils. *Journal of Plant Nutrition and Soil Science*, **174**, 576–586.

Jagadamma, S., Lal, R., Ussiri, D.A.N., Trumbore, S.E. & Mestelan, S. 2010. Evaluation of structural chemistry and isotopic signatures of refractory soil organic carbon fraction isolated by wet oxidation methods. *Biogeochemistry*, **98**, 29–44.

Joergensen, R.G. 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. *Soil Biology and Biochemistry*, **28**, 25–31.

Juma, N.G. & Paul, E.A. 1984. Mineralizable soil nitrogen: Amounts and extractability ratios. *Soil Science Society of America Journal*, **48**, 76–80.

Kader, M.A., Sleutel, S., Begum, S.A., D'Haene, K., Jegajeevagan, K. & De Neve, S. 2010. Soil organic matter fractionation as a tool for predicting nitrogen mineralization in silty arable soils. *Soil Use Management*, **26**, 494–507.

Kleber, M. & Johnson, M.G. 2010. Advances in understanding the molecular structure of soil organic matter: implications for interactions in the environment. *Advances in Agronomy*, **106**, 77–142.

Knicker, H. 2000. Biogenic nitrogen in soils as revealed by solid-state ^{13}C and ^{15}N NMR spectroscopy. *Journal of Environmental Quality*, **29**, 715–723.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry*, **34**, 139–162.

Leinweber, P. & Schulten, H.R. 2002. Non-hydrolyzable forms of soil organic nitrogen: extractability and composition. *Journal of Plant Nutrition and Soil Science*, **163**, 433–439.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. & Marschner, B. 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms *Soil Biology and Biochemistry*, **39**, 2183–2207.

Mikutta, R. & Kaiser, K. 2011. Organic matter bound to mineral surfaces: resistance to chemical and biological oxidation. *Soil Biology and Biochemistry*, **43**, 1738–1741.

Mikutta, R., Kleber, M., Torn, M. & Jahn, R. 2006. Stabilization of soil organic matter:association with minerals or chemical recalcitrance? *Biogeochemistry*, **77**, 25–56.

O'Connell, A.M. & Rance, S.J. 1999. Predicting nitrogen supply in plantation eucalypt forests. *Soil Biology and Biochemistry*, **31**, 1943–1951.

Olk, D.C. & Gregorich, E.G. 2006. Overview of the symposium proceedings "Meaningful pools in determining soil carbon and nitrogen dynamics". *Soil Science Society of America Journal*, **70**, 967–974.

Parfitt, R.L. & Salt, G.J. 2001. Carbon and nitrogen mineralisation in sand, silt, and clay fractions of soils under maize and pasture. *Australian Journal of Soil Research*, **39**, 361–371.

Ros, G.H. 2011. *Predicting soil nitrogen mineralization. Relevance of extractable soil organic matter fractions*. PhD thesis, Wageningen University, Wageningen, The Netherlands. Available at: http://edepot.wur.nl/168954; accessed 15/11/2012.

Ros, G.H. 2012. Predicting soil N mineralization using organic matter fractions and soil properties: a re-analysis of literature data. *Soil Biology and Biochemistry*, **45**, 132–135.

Ros, G.H., Hoffland, E. & Temminghoff, E.J.M. 2010. Dynamics of dissolved and extractable organic nitrogen upon soil amendment with crop residues. *Soil Biology and Biochemistry*, **42**, 2094–2101.

Schmidt, M.W.I. 1998. Organic matter in natural soils and in soils contaminated by atmospheric organic particles from coal processing industries. Dissertation. Ruhr-University Bochum, Bochum.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S. & Trumbmore, S.A. 2011. Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Schulten, H.R. & Leinweber, P. 1995. Dithionite-citrate-bicarbonate-extractable organic-matter in particle-size fractions of a haplaquoll. *Soil Science Society of America Journal*, **59**, 1019–1027.

Schulten, H.R. & Schnitzer, M. 1998. The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils*, **26**, 1–15.

Sharifi, M., Zebarth, B.J., Burton, D.L., Grant, C.A., Porter, G.A. & Cooper, J.M. 2007. Evaluation of laboratory-based measures of soil mineral nitrogen and potentially mineralizable nitrogen as predictors of field-based indices of soil nitrogen supply in potato production. *Plant and Soil*, **301**, 203–214.

Six, J., Bossuyt, H., Degryze, S. & Denef, K. 2004. A history of research on the link between (micro) aggregates, soil biota and soil organic matter dynamics. *Soil and Tillage Research*, **79**, 7–31.

Sleutel, S., Leinweber, P., Begum, S.A., Kader, M.A. & De Neve, S. 2009. Shifts in soil organic matter composition following treatment with sodium hypochlorite and hydrofluoric acid. *Geoderma*, **149**, 257–266.

Sleutel, S., Kader, M.A., Begum, S.A. & De Neve, S. 2010. Soil organic matter stability in sandy cropland soils is related to land-use history. *Journal of Plant Nutrition and Soil Science*, **173**, 19–29.

Sleutel, S., Leinweber, P., VanRanst, E., Kader, M.A. & Jegajeevagan, K. 2011. Organic matter in clay density fractions from sandy cropland soils with differing land-use history. *Soil Science Society of America Journal*, **75**, 521–532.

Springob, G. & Kirchmann, H. 2003. Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. *Soil Biology and Biochemistry*, **35**, 629–632.

Vance, E.D., Brookes, P.C. & Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Verstraete, W. & Voets, J.P. 1976. Nitrogen mineralization tests and potentials in relation to soil management. *Pedologie*, **26**, 15–26.

Zimmermann, M., Leifeld, J., Abiven, S., Schmidt, M.W.I. & Fuhrer, J. 2007. Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. *Geoderma*, **139**, 171–179.