Biogeosciences, 10, 7435–7447, 2013
www.biogeosciences.net/10/7435/2013/
doi:10.5194/bg-10-7435-2013

Biogeosciences
Open Access

Subcritical water extraction to isolate kinetically different soil nitrogen fractions

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Received: 2 May 2013 – Published in Biogeosciences Discuss.: 17 June 2013
Revised: 18 September 2013 – Accepted: 27 September 2013 – Published: 19 November 2013

Abstract. Soil organic N is largely composed of inherently biologically labile proteinaceous N and its persistence in soil is mainly explained by stabilization through binding to minerals and other soil organic matter (SOM) components at varying strengths. In order to separate kinetically different soil N fractions we hypothesize that an approach which isolates soil N fractions on the basis of bonding strength is required, rather than employing chemical agents or physical methods. We developed a sequential subcritical water extraction (SCWE) procedure at 100, 150 and 200 °C to isolate SOM fractions. We assessed these SCWE N fractions as predictors for aerobic and anaerobic N mineralization measured from 25 paddy soil cores in incubations. SCWE organic carbon (SCWE OC) and N (SCWE N) increased exponentially with the increase of temperature and N was extracted preferentially over OC. The efficiency of SCWE and the selectivity towards N were both lower in soils with increasingly reactive clay mineralogy. Stepwise linear regression found no relations between the SCWE fractions and the anaerobic N mineralization rate but instead with pH and a model parameter describing the temperature dependency of SCWE extraction. Both were linked to texture, mineralogy and content of pedogenic oxides, which suggests an indirect relation between anaerobic NH_4^+ release and these edaphic soil factors. N mineralization appeared to be largely decoupled from SOM quantity and quality. From the present study on young paddy soils low in pedogenic oxides and with high fixed NH_4^+ content we cannot infer the performance of SCWE to isolate bio-available N in more developed upland soils. There may be potential to separate kinetically different SOM pools from upland soils because 1° for aerobic N mineralization at 100–150 °C SCWE N was the best predictor; and 2° SCWE selectively extracted N over C and this preference depended on the mineralogical composition. Hence N fractions differing in bonding strength with minerals or SOM might be isolated at different temperatures, and specifically this association has frequently been found a prominent stabilization mechanism of N in temperate region cropland soils.

1 Introduction

Mineralization of soil organic nitrogen (N) remains a significant source of mineral N in paddy rice cultivation despite the widespread use of inorganic N fertilizers and organic N sources. Strikingly, often poor correlations were found between N mineralization in the field and general soil properties like N or organic carbon (OC) content and the soil's C : N ratio or a combination of those factors with pH, cation exchange capacity, clay content, etc. (Sahrawat, 1983a, b, 2006, 2010; Ros et al., 2011). Instead, successful indices to predict paddy soil's N supplying capacity include N uptake by plants (Sahrawat, 1983a; Nayyar et al., 2006; Curtin and McCallum, 2004) or measurement of increases in soil inorganic N upon incubation (Augus et al., 1994; Mikha et al., 2006). Yet, such "reference" biological methods are time-consuming and expensive with little significance for in-time fertilizer recommendation. Consequently, a multitude of simple and rapid chemical indices of paddy soil's N supplying capacity have been tested. For example, recently, Li et al. (2011) found that the soil's C : N ratio, acid $KMnO_4$-NH_4^+-N, alkaline $KMnO_4$-NH_4^+-N, phosphate-borate buffer extractable NH_4^+-N, phosphate-borate buffer hydrolysable NH_4^+-N and hot KCl extractable NH_4^+-N were all significantly ($P < 0.05$) correlated to total N uptake by rice plants. However, the relation between such chemical soil N fractions and N supplying processes actually occurring in the soil is weak and chemical approaches do not represent physical aspects of bio-availability of N. For instance, physical occlusion of particulate organic matter in microaggregates slows its microbial consumption (e.g. Six et al., 2000). In addition, soil structure controls microbial community structure and thereby indirectly determines soil N supply. For example, in soil with a larger proportion of 15–30 µm pore necks, Sleutel et al. (2011) found a promotion of fungi over bacteria. Since both decomposer groups have differing C : N ratios and N requirements, variation in microbial community structure logically results in differing utilization of OM and in mineral N release. In addition, chemical extractions generate artefacts, e.g. acid hydrolysis, aimed at removing labile organic matter, also liberates mineral bound OM by dissolution of Fe oxides at low pH (Kögel-Knabner et al., 2008). As a result, the development of a single chemical N index that is applicable to many types of soils, crops, and locations has not been successful (Wilson et al., 1994). In fact from an extensive meta-analysis Ros et al. (2011) concluded that the relationship between extractable OM and soil N supply is mostly an indirect relationship reflecting the soil's OM content.

Organic N is largely composed of inherently biologically labile proteinaceous N ranging between 40 % (Schulten and Schnitzer, 1998) and 80 % (Knicker, 2000), and a smaller share is present as heterocyclic N. Persistence of soil N is primarily explained by stabilization through binding to minerals and other SOM components and Leinweber and Schulten (2000), for example, revealed a substantial part of proteinaceous and heterocyclic N to be selectively bound to pedogenic oxides. Appelqvist et al. (1996), in addition, demonstrated linkages between amino acids and humic acids and based on extensive molecular modelling Schulten and Schnitzer (1997) suggested that proteinaceous materials can be trapped in the voids of the three-dimensional structure of humic substances. In an alternative view on the physicochemical organization of soil organic matter, Kleber et al. (2007) instead hypothesized that proteinaceous N is enriched close to mineral surfaces and shielded by an outer zone of more hydrophobic SOM constituents. Regardless of the conceptual model, variation in strength of N-mineral and N-OM linkages is likely to determine variation in bioavailability of organic N and susceptibility to mineralization. Physical fractionation methods are limited in their ability to further subdivide this continuum of bound organic N, because of practical constraints to subdivide the clay size fraction which, however, does contain the majority of soil N. Therefore an alternative approach, which isolates soil N fractions on the basis of bonding strength may be required, ideally then with respect to dissolvability into water instead of size or density or solubility in chemical agents. In addition, the release of "fixed" NH_4^+ is often an important crop supply of mineral N in paddy soils (Schneiders and Scherer, 1998), next to N mineralization. It has become clear that there also exists a continuum in the bio-availability of fixed NH_4^+ (Nieder et al., 2011), which is linked to its association with soil minerals. Commonly, a distinction is made between "native fixed" and "recently fixed" NH_4^+, where the former is probably trapped in the centre of clay interlayers to a higher degree, while the latter pool is largely retained in the peripheral zone of the interlayers (Nommik and Vahtras, 1982).

We hypothesize that soil N extracted by water at increasing temperatures would reflect organic and inorganic N fractions with increasing bonding strength, which in turn should correspond to decreasing bio-availability. Indeed, Kalbitz et al. (2005) amongst others concluded that strength of sorption reflects stability of the sorbed OM against biodegradation. Although water has frequently been used to quantify labile N or plant available N (e.g. Chantigny et al., 2010; and Curtin et al., 2006) or labile C (Sparling et al., 1998; Ghani et al., 2003) extraction of SOM has most often been limited to a temperature of 100 °C due to the difficulties of keeping it in a liquid state above 100 °C. This has limited the potential of water extraction to the isolation of readily bio-available SOM. Accelerated solvent extractors now conveniently offer the ability to extract soil samples with solvents at higher temperatures. By raising the inner pressure in metal extraction cells containing the soil sample, subcritical (100–375 °C) and supercritical (> 375 °C) water have been used to extract a variety of organic compounds from solid matrixes for chemical analysis (Ong et al., 2006; Lagadec et al., 2000). To our knowledge, SOM extraction by subcritical water has only been tested by Schnitzer et al. (1991) on four Canadian soils and recently, a custom-built instrument was developed for biomarker extraction in upcoming Mars explorer missions (Amashukeli et al., 2008). There appears to be no published information on the relation between subcritical water extractible N and OC and soil N mineralization. In the present study we developed a sequential subcritical water extraction (SCWE) procedure at 100, 150 and 200 °C to isolate SOM fractions from 25 paddy soil samples and we investigated how clay content and mineralogy affected extractability of N and C. Our main objective was to investigate the potential of SCWE N fractions to predict aerobic and anaerobic paddy soil N mineralization.

2 Materials and methods

2.1 Soils

A set of 25 paddy soils was previously collected (Kader et al., 2013) from agricultural fields throughout northern Bangladesh, which has a subtropical monsoon climate (annual mean temperature of 25.8 °C and annual precipitation of 2428 mm; Bangladesh Meteorological Department, 2011). Either one or two rice crops were cultivated per year on the sampled fields. Surface soil samples (0–15 cm) were collected from 15 locations per field by means of an auger (diameter 2.5 cm). These samples were bulked into one composite sample and were thoroughly mixed. The field's moist soil was gently broken apart by hand, air dried and ground to pass a 2 mm sieve prior to further analysis.

2.2 Analysis of soil properties, N mineralization and clay mineralogy

Soil texture, soil C and N content, pH in KCl (pH_{KCl}), NH_4-oxalate extractable Fe, Al and Mn (Fe_{ox}, Al_{ox}, Mn_{ox}) were determined on all 25 soils by Kader et al. (2013) (Table 1). Briefly, the soil texture of the collected soil samples ranged from sand to clay with most soils being silt loams. With an average pH_{KCl} of 4.5, all soils were moderately acidic (Table 1). There were wide ranges in C and N content with averages of $14.40 \pm 7.14\,\mathrm{g\,C\,kg^{-1}}$ and $1.48 \pm 0.66\,\mathrm{g\,N\,kg^{-1}}$. Ammonium oxalate extractable Fe also varied widely (1.1–8.3 $\mathrm{g\,Fe\,kg^{-1}}$), while variation in Al_{ox} and Mn_{ox} was limited.

Fixed NH_4^+ was determined by the Silva and Bremner method (Silva and Bremner, 1966), which consists of two steps. First, oxidation by alkaline KOBr removes exchangeable NH_4^+ and organic N. Then treatment with a 5 N HF-1N HCl solution dissolves clay containing fixed NH_4^+. The NH_4^+ released by the HF-HCl treatment was then determined by steam distillation.

N mineralization was assessed earlier by Kader et al. (2013) by monitoring the release of NH_4 and NO_3 under controlled aerobic and anaerobic conditions. Briefly, for each soil 42 PVC tubes (5 cm diameter) were filled with 200 g of dry soil, 21 (3 replicates × 7 dates) for aerobic and 21 (3 replicates × 7 dates) for anaerobic incubation. Soil moisture content was brought to 50 % water filled pore space for assessment of aerobic N mineralization and soils were oversaturated with a standing water level of 2 cm for assessment of anaerobic N mineralization. Every two weeks, soils were sampled destructively by removing the soil from one tube per replicate. The aerobic N mineralization rates were estimated by fitting a zero-order kinetic model to the soil mineral N data: $N(t) = N_0 + k_0 t$, 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 ($\mathrm{mg\,N\,kg^{-1}}$), and k_0 is the linear N mineralization rate ($\mathrm{mg\,N\,kg^{-1}\,day^{-1}}$). The anaerobic N mineralization data were best described with a first-order model: $N(t) = N_A(1-\exp(-k_1 t))$, with N_A the mineralizable N and k_1 a first-order rate parameter.

The clay fraction's mineralogical composition was identified by X-ray diffraction on oriented samples after (i) K and Mg saturation, (ii) glycerol solvation, and (iii) drying of the K saturated samples at 300 and 550 °C for 2 h, as described in detail by Kader et al. (2013).

2.3 Subcritical water extraction

Soils were sequentially extracted with pressurized (10,3–11,7 MPa) liquid water at 100, 150 and 200 °C with an ASE 350 Accelerated Solvent Extractor (Dionex, Amsterdam, the Netherlands). The extraction residue at each temperature step was utilized for the next extraction, so that the 150 and 200 °C extracts exclude the 100 °C and 100 + 150 °C extractable material respectively. We limited the upper extraction temperature to 200 °C as, previously, Schnitzer et al. (1991) found rapid thermal degradation of SOM components around 250 °C. Soil samples of 7 g were mixed with acid washed sand at a 1 : 2 weight ratio in 22 mL capacity Dionex stainless steel extraction cells (equipped with PEEK seal ring and Viton O-rings) to achieve an adequate permeability for perfusion of the subcritical water. Quartz filters (27 mm) were fitted at the bottom and top of each cell. Each extraction cycle consisted of different steps: filling of the cell with water, heating, stationary extraction (static cycle time: 5 min, number of static cycles: 1), rinsing the cell with fresh deionized water (rinsing volume 60 %), and finally purging (80 s) with N_2 gas. The extracts were collected in 60 mL glass vials and their volumes were determined by weight difference with the empty vials. At the end of the sequential extractions, the solid residues were removed from the cells and were oven dried at 105 °C (Fig. 1). Subcritical water extractions were carried out in twofold.

2.4 Carbon and nitrogen analysis

Total OC in the subcritical water extracts was determined with a Shimadzu TOC-V analyser (Shimadzu Corp., Tokyo, Japan) with IR detection following thermal oxidation. Total N concentrations in the extracts were determined with the alkaline persulfate oxidation method (Koroleff, 1983). Produced NO_3-N and NO_3-N already present prior to persulfate oxidation was measured with a continuous flow autoanalyser (ChemLab System 4). Capped 10 ml test vials, used as reaction vessels for persulfate oxidation, were centrifuged at 2000 rpm prior to continuous flow analysis. The OC and N content of the dried SCWE residues were determined by elemental analysis (Variomax CNS analyser, Elementar Analysesysteme, Germany). C and N expressed in $\mathrm{g\,kg^{-1}}$ present in the different SCWE fractions were calculated taking into account their of N and C contents, the extract volumes and the dry matter weight of the extracted residues.

Table 1. Soil properties of sampled agricultural fields in Bangladesh (data from Kader et al., 2013).

	Soil series	Soil type	Cropping pattern*	Soil particles (%)			Soil OC	Soil N	C : N ratio	pH_{KCl} (–)	Fixed-NH_4^+	NH_4-oxalate extractable ($g\,kg^{-1}$)		
				> 50 µm	2–50 µm	< 2 µm	($g\,kg^{-1}$)	($g\,kg^{-1}$)	(–)		($g\,kg^{-1}$)	Fe_{ox}	Al_{ox}	Mn_{ox}
1	Sonatala-1	Aeric Haplaquepts	R-F-R	4	76	20	21.4	2.1	10.4	5.6	0.22	5.1	0.9	0.4
2	Sonatala-2	Aeric Haplaquepts	R-F-R	13	73	14	16.3	1.7	9.6	5.2	0.21	4.9	0.8	0.3
3	Faridgonj	Aeric Haplaquepts	R-F-R	14	79	7	11.4	1.2	9.5	5.3	–	2.2	0.6	0.2
4	Noakhali	Arents	V-V-R	9	79	12	10.0	1.1	9.4	5.7	–	2.9	0.7	0.1
8	Silmondi-1	Aeric Haplaquepts	R-R/F-R	14	59	27	16.4	1.6	10.1	5.5	0.13	4.1	0.7	0.2
10	Ghatail	Aeric Haplaquepts	R-R/F-F	9	41	50	21.2	2.2	9.4	4.9	0.32	4.5	1.1	0.1
11	Balina	Mollic Haplaquepts	R-F-F	17	48	35	15.3	1.9	7.9	4.9	0.33	7.3	0.9	0.6
12	Melandoho	Aeric Fluvaquents	R-F-R	21	63	16	8.2	0.9	8.8	3.9	0.16	3.3	0.6	0.2
13	Tarakanda	Typic Fluvaquents	F-F-R	78	14	8	3.9	0.4	9.5	4.0	0.05	1.1	0.2	0.1
15	Gorargao	Typic Haplaquepts	R-F-F	18	39	43	30.5	3.2	9.6	4.6	0.26	3.7	1.2	0.2
16	Noadda-1	Ultic Ustochrepts	F-F-R	10	46	44	9.2	1.0	9.1	3.7	0.15	6.1	0.9	0.2
19	Dhamrai	Typic Haplaquepts	R-F-F	15	60	25	17.0	1.9	9.2	3.7	0.28	3.6	0.8	0.1
24	Gopalpur	Aquic Eutrochrepts	R-F-R	5	88	7	7.6	0.9	8.6	5.7	–	3.4	1.2	0.6
25	Silmondi-2	Aeric Haplaquepts	R/F-F-R	6	61	33	12.4	1.3	9.3	4.7	0.24	4.8	0.9	0.2
26	Sonatala-3	Aeric Haplaquepts	R-F-R	18	60	22	12.1	1.3	9.5	4.1	0.15	3.7	0.6	0.2
27	Gangachhara	Typic Haplaquepts	F-F-R	27	60	13	10.0	1.0	10.4	4.1	0.12	2.0	1.3	0.1
28	Ranisankail	Udic Ustochrepts	P-F/J-R	71	19	10	6.5	0.7	9.4	4.2	0.07	0.4	0.6	0.1
29	Amnura	Aeric Albaquepts	P/M-F-R	19	65	16	12.4	1.5	8.2	4.4	0.05	1.4	0.4	0.1
31	Pritimpasa	Typic Haplaquepts	F-F-R	17	48	35	13.2	1.4	9.6	3.9	0.19	4.3	0.7	0.2
32	Sulla	Typic Haplaquepts	R-F-F	10	31	59	17.3	2.4	7.2	3.7	0.38	8.3	1.3	0.2
33	Silmondi-2	Aeric Haplaquepts	R-F-R	28	52	20	10.7	1.2	8.6	4.3	0.12	3.9	0.6	0.2
35	Noadda-2	Ultic Ustochrepts	R-F-R	24	50	26	10.5	1.1	9.3	4.1	–	6.1	1.0	0.2
36	Kalma	Aeric Albaquepts	R-F-R	10	60	30	14.4	1.5	9.7	4.8	–	7.2	0.8	0.2
37	Karail	Cumulic Humaquepts	R-F-F	20	48	32	35.2	2.7	12.8	3.4	–	4.7	1.0	0.2
38	Sonatala-4	Aeric Haplaquepts	V-V-R	29	62	9	8.1	0.8	10.7	4.5	–	3.5	0.4	0.5

* R – rice, F – fallow, P – potato, V – vegetable, M – mustard, J – jute.

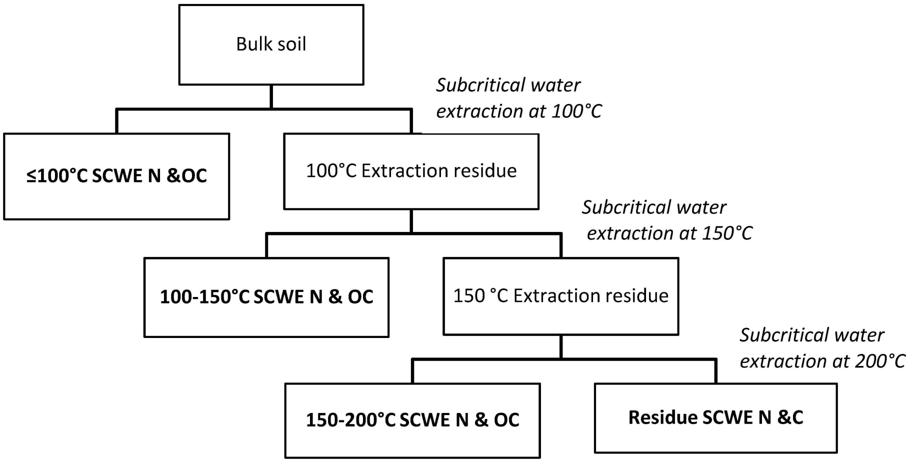

Fig. 1. Schematic of an experimental procedure for the sequential separation of subcritical water extracts. Extractions at 100, 150 and 200 °C were made by means of an accelerated solvent extractor.

2.5 Statistical analysis

Pearson's correlation coefficients were calculated to investigate the relation between N or C in the isolated SCWE fractions and the aerobic or anaerobic N mineralization rate. ANOVA (analysis of variance) was used to test for differences in N or C or the C : N ratio between groups of soils with different mineralogy. Non-linear regression with the Levenberg–Marquardt algorithm was used to estimate the first-order mineralization model parameters. For soils 4 and 24 the parameter N_A had to be constrained to a value below the soil N content and instead the sequential quadratic programming method was used. Multivariate linear regression was conducted with the N mineralization rates as dependents and the contents of OC and N of all the isolated fractions along with 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 $F < P_{\text{in}} = 0.05$) or removed (if $F > P_{\text{out}} = 0.10$). All statistical analyses were conducted with IBM SPSS statistics 21.0 (SPSS Inc., USA).

3 Results

3.1 Mineralogical composition

X-ray diffraction analysis revealed that all studied K and Mg-saturated clay fractions were composed of a variable admixture of 2 : 1 phyllosilicates (peaks at 1.00, 0.500, and 0.333 nm), kaolinite (0.713 and 0.357 nm), quartz (0.425 and 0.333 nm), feldspar (0.320 nm), goethite (0.418 nm) and lepidocrocite (0.627 nm). The broad bulge around 1.80 nm in the Mg-saturated and glycerol-solvated swelled specimens confirmed the presence of smectite in soils 4, 24 and 31. Mica was identified in all 25 soils by the presence of the 1.00 nm reflection appearing in all the treatments. Vermiculite was identified in 18 soils (not in soils 3, 8, 10, 15, 29, 31 and 37) by a decrease in the peak intensity of the 1.42 nm reflection and a corresponding increase in the peak intensity of the 1.00 nm reflection when shifting from Mg saturation to K saturation followed by air drying. Chlorite was detected in all soils except in soils 29, 35 and 37 by reflections at 1.42 nm in the K- and Mg-saturated air dried specimens and its rational orders and by the remains of the 1.42 nm reflection in the 550 °C-heated K-saturated specimen. Presence of kaolinite was confirmed in all studied soils from peaks or shoulders at 0.716 and 0.357 nm in the Mg-saturated specimens. In 14 soils, presence of a vermiculite-chlorite intergrade was ascertained by a decrease in peak intensity of the 1.42 nm reflection after heating the K-saturated specimen. Mostly, very small peaks at 1.25 nm in all the treatments also suggested the presence of the interstratified mica-chlorite mineral in 11 soils. Quartz and feldspars were identified in all soils, except for feldspars in soils 35, 37 and 38. Goethite and lepidocrocite were identified only in soils 1, 4, 12 and 36 and 10, 16 and 25, respectively. The X-ray diffraction clay mineralogy is summarized in Table 2.

3.2 N mineralization

The evolution of mineral N during 120 day incubations under aerobic and anaerobic conditions were described in detail by Kader et al. (2013). In summary, over the set of 25 soils the calculated aerobic N mineralization varied from 10 to 223 mg N kg^{-1} 120 days^{-1}, that is 2.5 to 15 % of the soil N was mineralized in 120 days, respectively). The anaerobic N mineralization varied from 34 to 423 mg N kg^{-1} 120 days^{-1} (4.5 to 28.1 % of soil N, respectively). Over all 25 soils, the mean anaerobic N mineralization rate was significantly higher (pairwise t test at $P < 0.05$) than the mean aerobic N mineralization rate.

3.3 SCWE fractions

Four different SOM fractions were obtained from sequential SCWE, namely ≤ 100, 100–150 and 150–200 °C water extractable and > 200 °C residual non-extractable N and C. The sizes of the N fractions were expressed on a bulk soil basis (g N kg^{-1}) and are given in Fig. 2. The recovery of N and OC was 90.6 ± 13.4 and 97.9 ± 7.2 % of soil N and soil OC, respectively. The lower recovery of N compared to OC might have been due to N volatilization during extraction at higher temperatures. At 100 °C SCWE removed 0.01–0.10 g N kg^{-1}, N representing 1.1–7.7 % of soil N (Fig. 2), in line with previously reported proportions of hot water extractable N according to Kader et al. (2010) (2–5 % of soil N), Curtin et al. (2006) (2–7.5 % of soil N) and Leinweber et al. (1995) (3–5 % of soil N). The amount of additional N extracted at 150 °C (additional since 100 °C extractable OM was already removed by the preceding extraction at 100 °C) ranged between 0.06 and 0.29 g N kg^{-1}, accounting for 7.5–25.2 % of soil N. Another larger share of 24–57 % of soil N was subsequently extracted at 200 °C (i.e. 0.18–1.34 g N kg^{-1}) (Fig. 2). The average C : N ratios of these three fractions decreased with increasing extraction temperature from 9.7 (≤ 100 °C SCWE fraction) over 8.4 (100–150 °C SCWE fraction) to 6.4 (150–200 °C SCWE fraction) (Fig. 3). Over all fractions, the ≤ 100 °C SCWE fraction's C : N ratio showed the largest variation (5–22) and did not significantly differ from the bulk soil's C : N ratio, in contrast to the 100–150 and 150–200 °C SCWE fractions. The SCWE residue N ranged between 0.09 and 1.41 g N kg^{-1} and represented the largest isolated soil N (24.2–63.1 % of soil N) fraction (Fig. 2). The C : N ratio of the extraction residues were logically higher (on average 15.8 ± 5.2) than the C : N ratios of the extracts and bulk soil (all at $P < 0.001$).

The temperature dependency of release of SCWE N and OC was quantified by fitting an exponential function: SCWE N or OC = $a\,e^{bT}$ to the cumulative amounts of N and OC (g kg^{-1}) at 100, 150 and 200 °C. T is the extraction temperature (°C), a is SCWE N or OC at $T = 0$ °C, and b is a constant expressing temperature dependency (°C^{-1}) of SCWE efficiency. This function fitted well to the SCWE-N and SCWE-OC data (average $R^2 = 0.99$ for N and OC), indicating an exponential increase of extractability of N and OC in response to subcritical water temperature increase from 100 to 200 °C (Fig. 4).

Among the 25 paddy soils, b varied between 0.018 and 0.032 °C^{-1} for SCWE N and between 0.016 and 0.028 °C^{-1} for SCWE OC. These values translate to relative increases in SCWE N and OC of 2–4 % and 1–3 % per °C increase in extraction temperature, respectively. The b parameter for OC correlated negatively to the sand percentage ($P < 0.05$), and

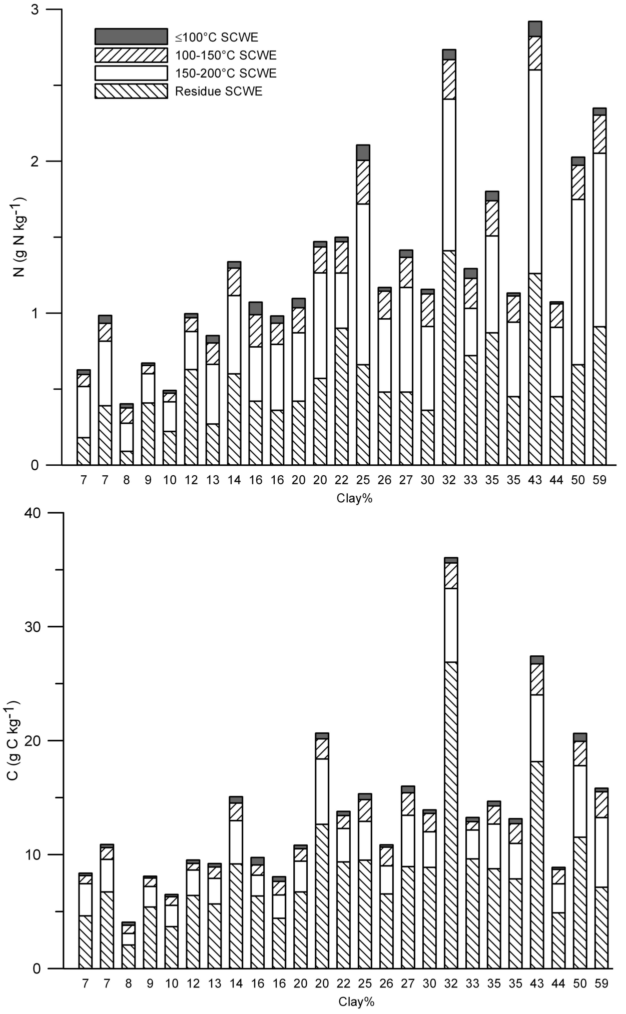

Fig. 2. Distribution of soil N and OC over subcritical water fractions. Fractions were subsequently extracted at 100 (≤ 100 °C SCWE N or OC), 150 (100–150 °C SCWE N or OC), 200 °C (150–200 °C SCWE N or OC), leaving an extraction residue (Residue SCWE N or OC).

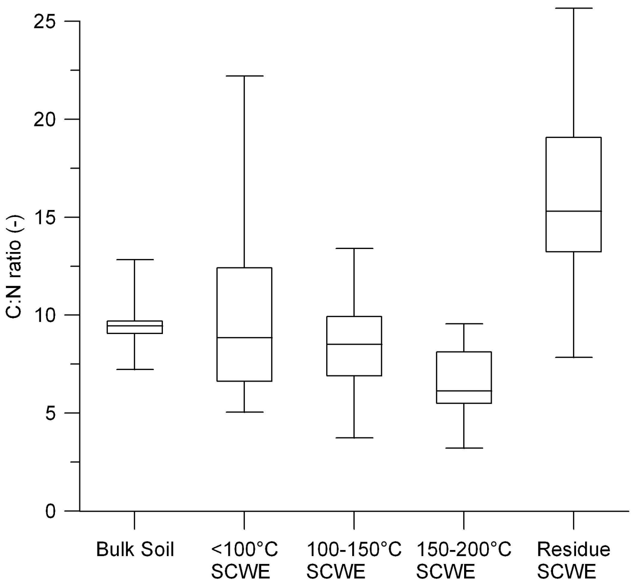

Fig. 3. Average C : N ratios of the bulk soil, subcritical water extracts and the extraction residue ($n = 25$, error bars show standard deviations).

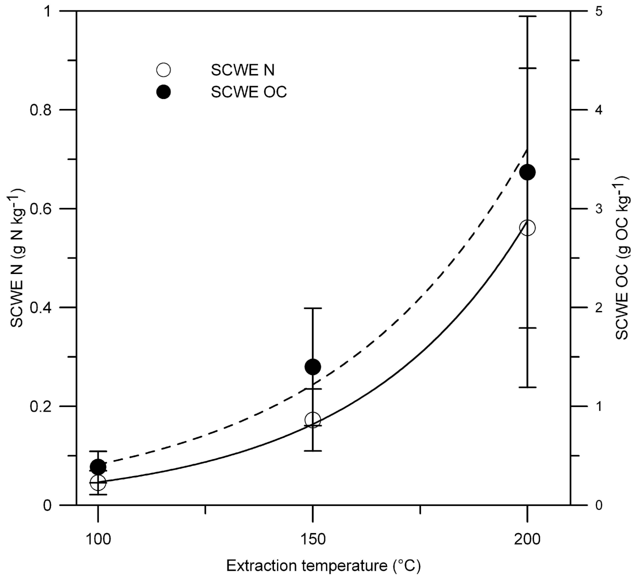

Fig. 4. Release of N and OC by subcritical water extraction in function of the extraction temperature. Symbols show means with standard deviations in error bars of 25 paddy soils, line graphs show exponential models fitted to the data.

to $\mathrm{Al_{ox}}$ ($P < 0.01$). The b parameter for N was only positively correlated to soil N.

3.4 SCWE N and C in relation to soil properties and clay mineralogy

Pearson's correlation coefficients were firstly calculated between SCWE N and SCWE OC and general soil properties (Table 3). All the isolated SCWE N and SCWE OC fractions correlated significantly and positively with soil N ($P < 0.01$) and soil OC ($P < 0.05$). Correlation coefficients generally increased with increasing extraction temperature. The 100–150 and 150–200 °C SCWE N and SCWE OC were strongly correlated with clay percentage, $\mathrm{Fe_{ox}}$ and $\mathrm{Al_{ox}}$, and fixed $\mathrm{NH_4}$-N content. On the contrary, ≤ 100 °C SCWE N and OC was not correlated with these soil parameters. SCWE residue OC was comparatively weaker ($P < 0.05$) correlated to these general soil properties.

In order to investigate if extractability of N and OC by means of SCWE depended on clay mineralogy, relative proportions of clay minerals were estimated by dividing the surface of each identified X-ray diffraction peak by the sum of all peaks in the X-ray diffraction spectra. A correlation

Table 2. Clay fraction mineralogical composition of sampled agricultural fields in Bangladesh (data from Kader et al., 2013).

ID	Soil Series	Mica	Smectite	Vermiculite	Chlorite	Kaolinite	Vt-Ch[a]	Mc-Ch[a]	Quartz	Goethite	Lepidocrocite	Feldspar
1	Sonatala-1	xxx[b]		x	xxx	xx		x	x	x		xx
2	Sonatala-2	xxx		xx	xxx	xx		x	x			x
3	Faridgonj	xxx			xxx	xx	xx		x			xx
4	Noakhali	xxx	x	xx	xxx	xx	x	x	x	x		x
8	Silmondi-1	xx			xxx	xx	xx	x	xx			xx
10	Ghatail	xx			xxx	xx	xx		xx		x	xx
11	Balina	xxx		x	xxx	xx			xx			xx
12	Melandoho	xxxx		x	xx	xx	x	x	x	x		x
13	Tarakanda	xxx		x	xx	xx	x	x	xx			xx
15	Gorargao	xxxx			xx	xx	x	x	xx			xx
16	Noadda-1	xxx		x	x	xx			xx		x	x
19	Dhamrai	xxxx		x	xxx	xx		x	xx			xx
24	Gopalpur	xxx	x	x	xx	xx	x		xx			xx
25	Silmondi-2	xxx		x	xxx	xx			xx		x	xx
26	Sonatala-3	xxx		xx	xxx	xxx			x			x
27	Gangachhara	xxxx		x	xxx	x		x	x			xx
28	Ranisankail	xxxx		x	xxx	x			x			x
29	Amnura	xxx				x	x		xx			x
31	Pritimpasa	xxx	x		xx	xxx			xx			x
32	Sulla	xxxx		x	x	x	x		xx			xx
33	Silmondi-2	xxx		x	xx	xx	xx	x	x			x
35	Noadda-2	xxx		x		xx	x		xx			
36	Kalma	xxxx		xx	xx	xx			xx	x		x
37	Karail	xxx				xx	x		xx			
38	Sonatala-4	xxx		x	xx	xx	xx	xx	x			

[a] Vt-Ch: vermiculite-chlorite interstratified minerals; Mc-Ch: Mica-chlorite interstratified minerals.

[b] Semi-quantitative analysis of clay fraction mineralogy based on X-ray diffraction patterns: x – indications of presence; xx – present; xxx – substantial presence; xxxx – abundant.

analysis between the approximate proportions of clay minerals and the relative proportions of SCWE N and OC (% of soil N or % of soil OC) showed that the proportion of ≤ 150 °C SCWE N was negatively correlated ($P < 0.05$) to the summed proportion of smectite and vermiculite and vermiculite-chlorite intergrade, while the summed proportion of 100–150 and 150–200 °C SCWE N was positively correlated with the proportion of quartz ($P < 0.05$). In addition, the observed decrease in the C : N ratio between the 100–150 and the 150–200 °C SCWE fractions was correlated to the proportion of kaolinite ($P < 0.01$). Similarly, the difference in the C : N ratio between the bulk soil and the 150–200 °C SCWE fraction was positively correlated ($P < 0.05$) with the proportion of quartz, but negatively ($P < 0.05$) with the proportion of vermiculite. This decrease in the C : N ratio was also significantly higher ($P < 0.05$) for soils containing no vermiculite or smectite (average decrease by 4.1) than for soils containing smectite (average decrease by 1.3).

3.5 SCWE N and OC and N mineralization

The N and OC in all the SCWE fractions and in the bulk soil was significantly correlated with the aerobic N mineralization rate but not with the anaerobic N mineralization rate (Table 4). The strongest correlation was found between the aerobic N mineralization rate and the sum of the ≤ 100 and 100–150 °C SCWE N fractions ($r = 0.70$). A stepwise linear regression was conducted between the aerobic or anaerobic N mineralization rates and the contents of SCWE N and OC fractions, general soil properties and the a and b parameters of the exponential model describing temperature dependency of cumulative C and N extraction by SCWE. Stepwise linear regression withheld only 100 + 150 °C SCWE N as a predictor variable ($R^2 = 0.49$; $P < 0.01$, $N = 25$) of aerobic N mineralization. In case of the anaerobic N mineralization both soil pH and the b parameter for SCWE OC were withheld ($R^2 = 0.54$; $P < 0.01$, $N = 25$), with positive coefficients (both at P≤0.01). The variance inflation factors of the excluded variables, 100–150 °C SCWE N and 150 + 200 °C SCWE N, were high (> 3) and all were strongly correlated to the 6% NaOCl-ox N. This demonstrates multicollinearity and redundancy between these predictor variables and the withheld predictor variable 100 + 150 °C SCWE N.

4 Discussion

4.1 SCWE

The ≤ 100 °C SCWE N constituted only but a few percent of total N, while about 40 % of soil N was extracted at 200 °C. This clearly demonstrates a much higher efficiency of SCWE compared to common "hot-water" extraction. The temperature response of SCWE was well described by an exponential

Table 3. Pearson's correlation coefficients ($n = 25$) between subcritical water extracted N and C ($g\,kg^{-1}$), contents of soil N, soil OC, ammonium oxalate extractable Fe and Al (Fe_{ox}, Al_{ox}), and fixed NH_4-N ($g\,kg^{-1}$) and the soil clay percentage (clay %).

	SCWE N				SCWE OC			
SOM fractions	≤ 100 °C	100–150 °C	150–200 °C	Residue	≤ 100 °C	100–150 °C	150–200 °C	Residue
Soil N	0.64**[a]	0.77**	0.92**	0.85**	0.69**	0.88**	0.91**	0.83**
Soil OC	0.56**	0.69**	0.83**	0.85**	0.64**	0.83**	0.88**	0.96**
Clay %	0.24	0.70**	0.73**	0.59**	0.33	0.74**	0.68**	0.41*
Fe_{ox}	−0.03	0.56**	0.46*	0.43*	0.00	0.54**	0.51**	0.26
Al_{ox}	0.22	0.42*	0.63**	0.43*	0.15	0.57**	0.64**	0.41*
Fixed NH_4-N[b]	0.33	0.72**	0.77**	0.74**	0.28	0.72**	0.78**	0.57*

[a] * Correlation is significant at $P < 0.05$ (2-tailed); ** Correlation is significant at $P < 0.01$ (2-tailed).

[b] ($n = 18$)

Table 4. Pearson's correlation coefficients between amounts ($g\,kg^{-1}$) of SCWE N and OC and the rate of soil N mineralization, measured under aerobic and anaerobic conditions.

	N mineralization rate ($mg\,N\,kg^{-1}\,day^{-1}$)	
Soil fraction	Aerobic	Anaerobic
Soil N	0.64**	0.24
Soil OC	0.60**	0.33
≤ 100°C SCWE N	0.57**	0.10
100–150 °C SCWE N	0.68**	0.35
150–200 °C SCWE N	0.58**	0.30
SCWE Residue N	0.44*	0.33
100 + 150 °C SCWE N	0.70**	0.30
150 + 200 °C SCWE N	0.61**	0.32
≤ 100 °C SCWE OC	0.44*	−0.23
100–150 °C SCWE OC	0.48*	0.32
150–200 °C SCWE OC	0.49*	0.21
SCWE Residue OC	0.50*	−0.04
100 + 150 °C SCWE OC	0.51**	0.22
150 + 200 °C SCWE OC	0.50*	0.25

* Correlation is significant at $P < 0.05$ (2-tailed) and ** correlation is significant at $P < 0.01$ (2-tailed).

model (Fig. 4), consistent with Martinez et al. (2003), who found a nearly doubling of water extractable N and OC when the extraction temperature was raised from 10 to 50 °C and a nearly triple increase between 50 and 90 °C. The observed relative increases of SCWE N and OC per °C extraction temperature were also in line with findings of Chantigny et al. (2010), who reported a relative increase in water extractable OC of 1.4–5.3 % per °C increase within a gradient from 20 to 80 °C. Organic macromolecules may change from a rigid "glassy" state to a more flexible "rubbery" condition as temperature increases (Zhang et al., 2007) along with a decreasing viscosity of humic substances (Boutaric and Thenevet, 1937) and it is possible that this increases the water solubility of humic materials. However, changing physical properties of the solvent itself, in this case water, with temperature could have also determined extraction of soil N by SCWE. Water is a very polar solvent with a dielectric constant of 78.4 at 25 °C and 0.1 MPa and this limits the potential to extract important non-polar organic N components like non-polar amino acids (e.g. valine, alanine, leucine, methionine, and proline) and many proteins. However, as water's temperature and pressure increase, the thermal energy excess disorients individual molecular dipoles and lessens the strong dipole–dipole electrostatic interactions within the liquid, which results in lower dielectric constants (Fernandez et al., 1997). For example, the dielectric constant of water decreases to 31.5 at 225 °C and 20 MPa, which is very close to the dielectric constant of a less polar organic solvent like methanol (32.6 at 25 °C and 0.1 MPa) (Wohlfarth, 1991). Under these circumstances most lipids and heterocycles should still be insoluble. The gradual lowering in C : N ratio from 9.7 for the ≤ 100 °C over 8.4 at 100–150 °C to 6.4 at 150–200 °C fractions indeed revealed an increasing selectivity to N over OC (in more hydrophobic substances) with increasing SCWE temperature. Gregorich et al. (2003) observed that the C : N ratio of water-extracted SOM was decreased with increasing extraction temperature from room temperature to 80 °C. They suggested that hot water facilitates the release of hydrophilic organic N. Schnitzer et al. (1991) also found a more efficient extraction of organic N than of other OM components at 200 °C. They attributed the greater affinity of subcritical water at 200 °C to the greater polarity of many N containing compounds, compared with carbohydrates and lignin- and phenol-derived aromatics. In view of these observations, it appears that by "tuning" of the dielectric constant through choice of SCWE temperature one can isolate OM fractions with differing solubility of N and OC in water.

Next to the temperature dependency of solubility of SOM in water, variation in strength of OM–mineral interactions most probably governs the extractability of soil N and C at different temperatures. In line with this, we expected clay content and mineralogy to determine SCWE efficiency and its dependency on temperature. Firstly, the significant positive correlations between the SCWE temperature dependency model parameter *b* for OC and clay percentage and Al_{ox} and Mn_{ox} confirm this for OC. In other words, higher extraction temperatures are required to extract equal amounts of OM from soils with higher content of clay or pedogenic oxides. Additionally, the data suggest that extraction of mineral bound N and OC primarily occurred at extraction temperatures above 100 °C as only the 100–150 and 150–200 °C, but not $\leq 100\,°C$ SCWE N and OC, correlated positively with the soil clay percentage and contents of Fe_{ox} and Al_{ox}. Similarly, fixed NH_4-N did not correlate to $\leq 100\,°C$ SCWE N, indicating that fixed NH_4-N was extracted primarily by SCWE above 100 °C. Secondly, the proportions of smectite and vermiculite correlated negatively with SCWE N, while the proportion of quartz was positively correlated. There was furthermore a negative correlation between *b* for N and the summed proportion of 2 : 1 clay minerals (though only at $P = 0.075$). From this we conclude a lower efficiency of SCWE in soils with increasingly reactive clay mineralogy. In addition, the increasing selectivity of SCWE towards N over C at higher temperatures, as indicated by the shift in C : N ratio in the SCWE fractions, depended on clay mineralogy as well. SCWE was more selective towards N with decreasing proportion of high surface area clay minerals like smectite and vermiculite.

To interpret these data, an analogy could be made to data obtained by pyrolysis field ionization mass spectroscopy, in which the temperature dependent release of individual OM molecular markers is resolved. Schulten and Leinweber (1999) found that volatilization of mineral bound OM requires higher temperatures than unbound OM to enable thermal bond cleavage. They identified three thermal classes: (i) unbound undecomposed plant fragments relatively rich in aliphatics; (ii) a thermally labile fraction containing N-containing compounds and carbohydrates associated with humified OM and (iii) thermally stable mineral bound OM. On the basis of the data in this study it seems plausible to hypothesize that SCWE at 100 °C primarily releases weak or intermediately bound N and OC, i.e. belonging to the second SOM pool identified by Schulten and Leinweber (1999). The first pool may well be of lesser importance because unbound undecomposed OM in fact comprises but a relatively small share of the soil N and is probably poorly extractable due to its particulate nature. Leinweber et al. (1995) found hot water extractable OM to be largely composed of carbohydrates and N containing compounds and since many of these constituents volatilized at lower temperatures during pyrolysis, it was concluded that they were in soil solution or just weakly sorbed to mineral surfaces or humic macromolecules. It seems acceptable that such material formed a major part of the SCWE fractions. At 150 and 200 °C, SCWE probably extracted increasingly more OM, which is strongly bound to minerals, i.e. belonging to the third pool defined by Schulten and Leinweber (1999). This was indirectly confirmed by correlation analysis with the clay percentage and contents of Fe_{ox} and Al_{ox}, as described above. Yet, direct supporting evidence should come from SCWE extraction of well known model OM–mineral mixtures. In addition, the average C : N ratio of the 150–200 °C SCWE fraction was 6.4, i.e. close to commonly observed clay fraction C : N ratios. This again suggests that OM bound to clay minerals or to pedogenic oxides was extracted by SCWE at 200 °C. As selectivity of SCWE towards N over C increased with decreasing proportions of smectite and vermiculite, it appears that extraction of N bound to such highly reactive minerals may have been limited.

4.2 SCWE N and C and N mineralization

The range in soil N ($0.4–3.2\,g\,kg^{-1}$), clay content (7.4–58.7 %), crop rotation (single or double annual rice crops) and mineralogy (Table 1) allowed a robust and meaningful evaluation of the SCWE N fractions as indices for N mineralization in paddy soils. This variation in the sampled soil set was also accompanied by a wide variation in the aerobic and anaerobic N mineralization rates.

The mean correlation coefficient *r* between soil N content and the aerobic N mineralization rate was 0.64 (Table 4), indicating that soil N content generally explained only 41 % of the observed variation in the N mineralization rate. Several researchers found the quality of soil N, as quantified by a distribution of N over physicochemical fractions or by the biochemical composition of SOM, to have a greater influence on mineralization of N than soil N or soil OC content (Yonebayashi and Hattori 1986; Olk et al., 1996, Cassman et al., 1996). For the 25 studied soils, however, only slightly stronger correlations were found with the 100–150 °C SCWE N ($r = 0.68$) and with the sum of the ≤ 100 and 100–150 °C SCWE N ($r = 0.70$) (Table 4). The resulting percentages of explained variance (46–49 %) are still too small to allow accurate fertilizer recommendations according to the guidelines given by Malley et al. (2004), who suggested that R^2 values of calibrated soil tests should be more than 83 % (Ros, 2012). Surprisingly, $\leq 100\,°C$ SCWE N had a weaker correlation with the aerobic and anaerobic N mineralization rates than soil N, although hot water extractable N (mostly at 80–100 °C) has been widely used as an index of plant available N (Broner and Bachler, 1980; Ghani et al., 2003; Curtin et al., 2006) and is considered to be composed of bio-available N-containing compounds (Landgraf et al., 2006). Unexpectedly, the sequential SCWE residue N correlated positively to the aerobic N mineralization rate. This relationship may have been indirect, though, because SCWE residue N was also strongly correlated to soil N content (Table 3) and constituted the largest isolated soil N fraction.

Finally, we assessed combinations of the isolated N and OC fractions and general soil properties as predictors of the N mineralization rate through multivariate regression, but explained variances remained low with a maximal R^2 of 0.54 for anaerobic N mineralization. We found no significant correlations at all between N or OC in any of the isolated soil fractions and the anaerobic N mineralization rate. Instead, soil pH and the SCWE OC temperature dependency model *b* parameter were the best predictors of anaerobic N mineralization, with negative and positive linear regression coefficients, respectively. The latter suggests, surprisingly, that N mineralization was higher in soils for which higher temperatures are required to extract soil OC, i.e. with a higher value of *b*. It would appear, however, that this relation was in fact indirect as at the same time *b* for OC correlated positively to $\mathrm{Al_{ox}}$ and $\mathrm{Mn_{ox}}$ and negatively to the sand percentage. In line, soil pH correlated positively to the silt percentage and negatively to the quartz percentage and $\mathrm{Mn_{ox}}$. Both predictors (pH and *b*) therefore appear to depend on soil texture and content of pedogenic oxides. This designates that SOM quality, at least when expressed as a distribution of N and C over isolated SCWE fractions, does not control the anaerobic N mineralization process at all and instead abiotic factors are of importance. In a previous study by Kader (2012) it was found that the N mineralization of these soils collected from agricultural fields was also not explained by content of N in different physicochemical fractions, including sand N, silt + clay N, 6 % NaOCl oxidizable N and 10 % HF-extractable N. Instead Kader et al. (2013) suggested that abiotic factors like availability of reducible Fe, mineralogy and pH could play a decisive role, but this needs to be confirmed through further research. In line, Sahrawat and Narteh (2003) found reducible Fe to be a useful indicator to predict ammonium production in 15 west African rice soils. Most of the sampled Bangladeshi paddy soils are in a fairly early stage of soil development with relatively low content of pedogenic oxides and active clay mineralogy. Both aspects could be of importance to mineral N release under anoxic conditions in the studied soils. Firstly, as redox potential progressively lowers after onset of submergence electron acceptors alternative to rapidly depleted O_2 and NO_3, viz. Mn^{4+} and Fe^{3+}, are required for OM decomposition to proceed. The availability of both would be limited in soils having a low pedogenic oxide content, and this may form a bottleneck for anaerobic N mineralization. Secondly, vermiculite containing paddy soils accumulate fixed NH_4^+ (estimated to 100–800 $\mathrm{kg\,N\,ha^{-1}\,15\,cm^{-1}}$ in the studied soils). It has, however, often been shown that a part of the fixed NH_4-N is in fact dynamic (Nieder et al., 2011) and therefore release of fixed NH_4^+ may well have contributed to build-up of exchangeable NH_4^+ during the anaerobic lab incubations. It has also been shown that microbial reduction of Fe^{3+} followed by dissolution of Fe-oxides coated on the surfaces of clay minerals is a prerequisite for fixed NH_4^+ exchange (Scherer and Zhang, 1999). Following these two points, again abiotic factors like clay mineralogy and content of pedogenic oxides, rather than SOM quality, should be of importance to the net-release of exchangeable NH_4^+ under anaerobic conditions in young paddy soils, in line with our regression analysis. In conclusion, quality and quantity of N fractions only partly explained the aerobic N mineralization and not anaerobic N mineralization of the studied set of 25 paddy soils and consequently alternative driving factors than the soil fractions isolated here should be looked for. The biochemistry of paddy soils is, however, controlled by complex interactions between redox potential, pH, solubility of OM (Kögel-Knabner et al., 2010) and availability of electron acceptors (Sahrawat, 2004).

Maybe we should not discard SCWE as a means for isolation of N pools with differing proneness to microbial decay, solely on the basis of this particular study on paddy soils. Bio-availability of N, approximated here by extractability of N at different temperatures, may well play a more important role in well-drained upland agricultural soils. Perhaps SCWE holds potential to separate kinetically different SOM pools in these soils. Indeed, the aerobic N mineralization did relate to several > 100 °C SCWE N fractions, although still with but a limited higher predictive power than soil N content. Under aerobic conditions, microbial activity is no longer dependant on availability of alternative electron acceptors and instead, as revealed by several authors (Appelqvist et al., 1996; Schulten and Schnitzer, 1997; Leinweber and Schulten, 2000; Kleber et al., 2007), association of organic N with soil mineral components is the dominant mechanism explaining the persistence of these inherently biologically labile SOM constituents. Our study, in line with Schnitzer et al. (1991), demonstrated that N is preferentially extracted over C with increasing temperature by SCWE and that the extraction efficiency depended on soil mineralogy. This suggests that SCWE at different temperature intervals may be able to isolate specific OM- or mineral-associated N fractions, which are likely to be differently bio-available. In light of mineral association of N, an important difference between young floodplain paddy soils and more developed upland soils may be their contents of pedogenic oxides, as these have been identified as specific important binding sites for organic N in sand to loam textured croplands in Germany (Leinweber and Schulten, 2000). Since the content of pedogenic oxides was low in the studied set of paddy soils we were not able to test the potential of SCWE to extract differently bound N pools that would be relevant to upland soils. It would be most interesting to see if and how SCWE is able to separate OM-associated N, pedogenic oxide bound N, NH_4^+ in clay mineral interlayers and N bound to clay minerals.

5 Conclusions

This study shows that SCWE can be used to preferentially extract N from soil and that the quantity of extracted N increases exponentially with the increase of temperature. The data suggested that extraction of mineral bound N and OC primarily starts above 100 °C and increases with temperature as well. Clay mineralogy also seems to significantly affect the extractability of N and OC by SCWE. Eventually, at 200 °C probably mostly clay fraction mineral bound OM is released by SCWE as seen from the negative relation between the extracts' C : N ratio and extraction temperature (to only 6.4 at 200 °C). We hypothesize that the stepwise SCWE at 100, 150 and 200 °C SCWE incrementally removed OM that is more strongly mineral bound and less OM that is weakly associated to mineral matter or SOM. If so, the three isolated SCWE fractions would each form mixtures of SOM pools with differing thermostability and perhaps also biodegradability. However, it seems that the sequential SCWE procedure was in fact unable to completely isolate kinetically labile from stable (mineral bound and/or biochemically recalcitrant) organic N, as the amount of N in each of the SCWE fractions and in the SCWE residue correlated with the aerobic N mineralization rate, indicating the presence of labile N in all of them. Alternatively, relations between N mineralization and SCWE extracted N may have been indirect since mutual positive correlations existed with soil N content. The present investigation of the SCWE procedure was conducted on a set of paddy soils, in which N mineralization seemed to be decoupled from SOM quantity or quality. Perhaps SCWE does, however, hold potential to separate kinetically different SOM pools in upland soils where the bio-availability of N is likely a key constraint in the N mineralization process.

Acknowledgements. MA Kader wishes to acknowledge the Flemish Inter-University Council (VLIR) for providing him a Ph.D. grant to carry out this research. S. Sleutel is working as a postdoctoral researcher for the Research Foundation Flanders (FWO). This research was partly funded by FWO grant 1.5.088.10N. The FWO is also acknowledged for the funding of the ASE equipment (1.5.062.09N).

Edited by: S. Bouillon

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