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High N relative to C mineralization of clover leaves at low temperatures in two contrasting soils

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ABSTRACT

Predicting N mineralization from green manure in different soil types during the cold season is instrumental for improving crop management with higher N use efficiency and reduced risks of N losses in a cool and humid climate. The objective of our work was to study the effects of low temperatures and soil type on the net nitrogen (N) mineralization and the relationship between N and carbon (C) mineralization from N-rich plant material. A silty clay loam and a sandy loam were incubated with or without clover leaves for 80 days at 0, 4, 8.5 or 15 °C. The results showed a substantial mineralization of N in clover leaves (7% of N added), unaffected by temperature, already on 3rd day. This was followed by net N immobilization for about 4 weeks in the clay soil, with similar tendencies in the sandy soil, and more severely at the higher than the lower temperatures. After 80 days of incubation, net N mineralization was only 13–22% of total N in clover leaves. The ratio of net mineralized N to C was higher at lower temperatures, and higher in the sandy than in the clay soil. After the immobilization period, the N mineralization increased, positively related to temperature, and the ratio of net mineralized N to C became constant. In conclusion, low temperature during the initial phase of mineralization altered the ratio between net N and C mineralization from easily decomposable plant material, and the net N mineralization occurred more rapidly in the sandy soil. The change in stoichiometry at low temperatures, as well as the modifying effect of soil type, should be considered when predicting N mineralization of N-rich plant material.

1. Introduction

In the absence of animal manure in stockless organic farming, green manure is grown to improve soil fertility, and thereby enhance the yield of subsequent cash crops. A wide range of legumes and non-legumes are used as green manure crops, for their nitrogen (N) supply or other benefits (Cherr et al., 2006). In cold temperate regions, clover is commonly used as green manure in organic arable farming, often in mixture with grasses, cultivated as an undersown catch crop in cereals or as a full-season green manure ley (Løes et al., 2011). Such leys are mown about three times by for example a pasture chopper and the herbage is left on-site as mulch. Then, the green manure ley is usually incorporated in the soil by ploughing in late autumn or in the following spring. The decomposing N-rich plant material is thus exposed to low temperatures during its initial turnover, before the establishment of the following crop. Despite the low temperatures, field studies have shown rapid N mineralization from such plant material (Breland, 1994; Thorup-Kristensen and Dresbøll, 2010).

In spring cereals, the presence of a sufficient amount of plant available N early in the growing season is a key factor for grain yield. However, the inorganic N present in the soil in the late autumn, winter and spring is, especially when the soil it not frozen, at risk of being lost through nitrate leaching, gaseous losses or runoff (Chantigny et al., 2002). The understanding of N mineralization during the cold season is therefore instrumental for predicting the fate and rate of N mineralizing from newly incorporated plant material, and thus for planning better

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crop management with higher N use efficiency and reduced risks of N losses to the environment.

N mineralization is closely linked to C mineralization, but at low temperature, the ratio of these processes has been found to be altered; C mineralization is depressed more than net N mineralization (Kirschbaum, 1995; Magid et al., 2001; Schütt et al., 2014). The suggested reason is that gross microbial growth (immobilization) is more impaired by low temperature than is gross N mineralization (Andersen and Jensen, 2001; Magid et al., 2001).

The decomposition of organic matter and the mineralization of its N content depend on the substrate and the environmental conditions, including the soil organisms (Swift et al., 1979). In general, decomposition is found to be more affected by temperature in the lower temperature range (<10 °C) than at higher temperatures (Kirschbaum, 1995; Kätterer et al., 1998). According to kinetic theory, the decomposition of substrate with high molecular complexity, i.e. soil organic matter, is more sensitive to temperature than is substrate with low molecular complexity, i.e. fresh plant material (Davidson and Janssens, 2006). Several studies confirm this, but contradictory results are also found (Giardina and Ryan, 2000; Fang et al., 2005). The variability of the response to temperature of different substrates is large, but discrepancies in the definition of the temperature effect, the experimental setting and the method of analysis may also affect the conclusions (Conen et al., 2008; Frøseth and Bleken, 2015).

Different soil types provide contrasting environments for decomposition of organic matter. Mineral particles and soil aggregates act as chemical and physical protection of organic substrates and microorganisms, and clay soils show slower decomposition rates not only of soil organic matter, but also of added readily decomposable substrate, than do sandy or silty soils (Van Veen et al., 1985; Saggar et al., 1996; Frøseth and Bleken, 2015). There is little knowledge about the effect of soil type on the N dynamics of decomposing N-rich plant residue, but it might be expected that N mineralization, similar to carbon (C) mineralization, is affected by soil type. Clark et al. (2009) incubated a clay and a loamy soil with organic amendments at –6 to 10 °C and found that the mineralization and nitrification rates were higher in the clay soil than in the loamy soil, especially below freezing point, which they suggested to be due to more unfrozen water remained in the clay soil. In a field trial, however, Müller and Sundman (1988) found that soil type varying from sandy loam to clay only slightly affected the release of clover N from mesh bags during Finnish winter conditions. Comparing the results of a Norwegian field trial at four locations, we found that following incorporation in early spring of a green manure as the only fertilizing input, the early N supply to a subsequent spring cereal was more deficient in clay than in sandy soil (Frøseth et al., 2014).

With the aim of acquiring more empirical data to understand the reason for the effect of soil type on the ability of green manure to supply mineral N in early spring, we investigated the decomposition of clover leaves in two contrasting soil types at low temperatures down to 0 °C. A detailed assessment of the effect of temperature and soil type on C mineralization is given in Frøseth and Bleken (2015). The results indicated that the mineralization of C from newly incorporated clover leaves was influenced by soil type and it occurred also at 0 °C. In the present article, we present the effect of temperature and soil type on the net N mineralization and the relationship between net N and C mineralization. Our hypotheses were:

(i) Considerable N mineralization from N-rich plant material occurs even at 0 °C.
(ii) The ratio of net mineralized N to mineralized C from N-rich plant material is larger at lower than at higher temperature.
(iii) The ratio of net mineralized N to mineralized C from N-rich plant material is not affected by soil type.

2. Materials and methods

2.1. Experimental design

The incubation experiment for measuring C and N mineralization ran for 80 days with two soil types with or without clover leaves, in chambers maintained at constant temperatures of 0, 4, 8.5 or 15 °C. The soils were taken from two arable fields, about 4 km apart, of the Kvithamar research center in Central Norway (63°29′N, 10°52′E). At this site, the normal (1961–1990) annual precipitation is 896 mm and the mean monthly soil temperature at 10 cm depth through the year (2006–2013) varies from –1 °C to 15 °C. The soils were a silty clay loam classified as Mollic Gleysol and a sandy loam classified as Arenic Fluvisol (IUSS Working Group WRB, 2006). The soils were not calcareous and did not contain carbonates. Further soil characteristics are available in Table 1.

Bulk samples of the soils were collected in spring 2011 from 0 to 20 cm depth, sieved through a 2 mm mesh while moist and pre-incubated in the dark for 4 ½ months at about 15 °C under aerobic and moist conditions. The soil was kept moist to minimize the disturbance of microorganisms and the sieving was done to remove gravel and homogenize the soil. Two and a half weeks prior to incubation the soil was further moistened to 75% of pore volume at field bulk density, which was about the highest moisture content that allowed handling and compaction of the soil without destroying the small aggregates. Before starting the incubation, the soil was kept at the final temperature for three days. Then the soil was filled in the incubation chambers. In half of the chambers, red clover leaves (*Trifolium pratense* L.), corresponding to 4 g dry matter kg^{-1} dry soil, were gently mixed. The soil was compacted to nearly the same bulk density as observed in the field (Table 1). The clover leaves contained 46.3% C and 4.8% N (C/N = 9.8). The amount corresponded to about 43–58 g N m^{-2} and 900–1200 g dry matter m^{-2} to 20 cm depth, which is roughly equal to the expected total aboveground dry matter production of a green manure ley during a single growing season (Frøseth et al., 2014). The clover leaves had been dried at 60 °C, cut into pieces, and sieved through a 2 mm mesh. The leaves were enriched in ^{13}C. Details about this enrichment are given by Frøseth and Bleken (2015).

There were two sets of incubation chambers. One set for the analysis of mineralized N (NO_3–N and NH_4–N), consisted of 50 g soil (dry weight) samples in 200 mL plastic cups, with lids open to allow some aeration.

A second set, for the analysis of CO_2 release, consisted of 400 g soil (dry weight) samples kept in 1 L DURAN® glass bottles with 2 M NaOH lye traps for collecting CO_2 (see Frøseth and Bleken, 2015).

Both sets of chambers were equally distributed between four incubators regulated at 0 °C (Refritherm 200, Denmark), 4 °C (Termaks KB 8182, Norway), 8.5 °C (Termaks KBP 6151, Norway) and 15 °C (Termaks KBP 6151, Norway). Temperature data loggers monitored the incubator temperature. The standard error for the incubator temperatures was ±0.2–0.3 °C. Within each temperature, soil samples with and without plant material, were replicated three times for gas analyses and four times for mineral N analyses.

2.2. Sampling and analysis

Samplings were performed within the first 20 h (day 0), and on days 3, 8, 15, 30, 52 and 80 after incubation started.

The chambers for inorganic N analysis were sampled destructively. The whole sample was extracted with 130 mL 2 M KCl, and analysed by spectrophotometry on a FIAstar™ 5000 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO_3–N and NH_4–N, respectively). Four samples of dry clover leaves without soil were also extracted and analysed by the same method to find the amount of inorganic N released from the leaves.

The lye in the CO_2 trap was collected in 20 mL pre-evacuated glass vials, from which 1 mL was transferred to a 10 mL glass vial prefilled

Table 1
Soil particle size distribution, bulk density, available water (10–1500 kPa), wilting point (1500 kPa), pH, total C and N in undisturbed soil, 0–20 cm.

Soil type	Sand % of fine earth	Silt	Clay	Bulk density g cm^{-3}	Available water vol%	Wilting point vol%	pH[a] -	Total C[a] %	C/N[a] -
Silty clay loam[b]	3	70	27	1.13	36	15	6.0	4.45	11.4
Sandy loam[c]	51	43	6	1.53	27	6	6.2	1.30	11.8

[a] Analysed in new samples.
[b] From profile no. 6 in Sveistrup et al. (1994).
[c] From profile 20,177 in Solbakken (1987).

with 1.5 mL 2 M H_2SO_4. This was used for determination of CO_2 on a gas chromatograph (GC) (Model 7890A, Agilent, Santa Clara, CA, US). The fractional abundance of ^{13}C was analysed with a gas chromatograph isotope ratio mass spectrometer (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany). In addition, at adequate intervals, the atmosphere of the gas tight glass chambers was sampled (5 mL injected into pre-evacuated 10 mL glass vials) and immediately analysed on the GC to monitor its O_2 content. Oxygen was added when necessary to increase the level to around 20% volume. The oxygen content was usually above 12%, with a few exceptions at the beginning of the trial.

The amount of total C and N in the soil and clover leaves was analysed using the Dumas combustion method (Bremmer and Mulvaney,

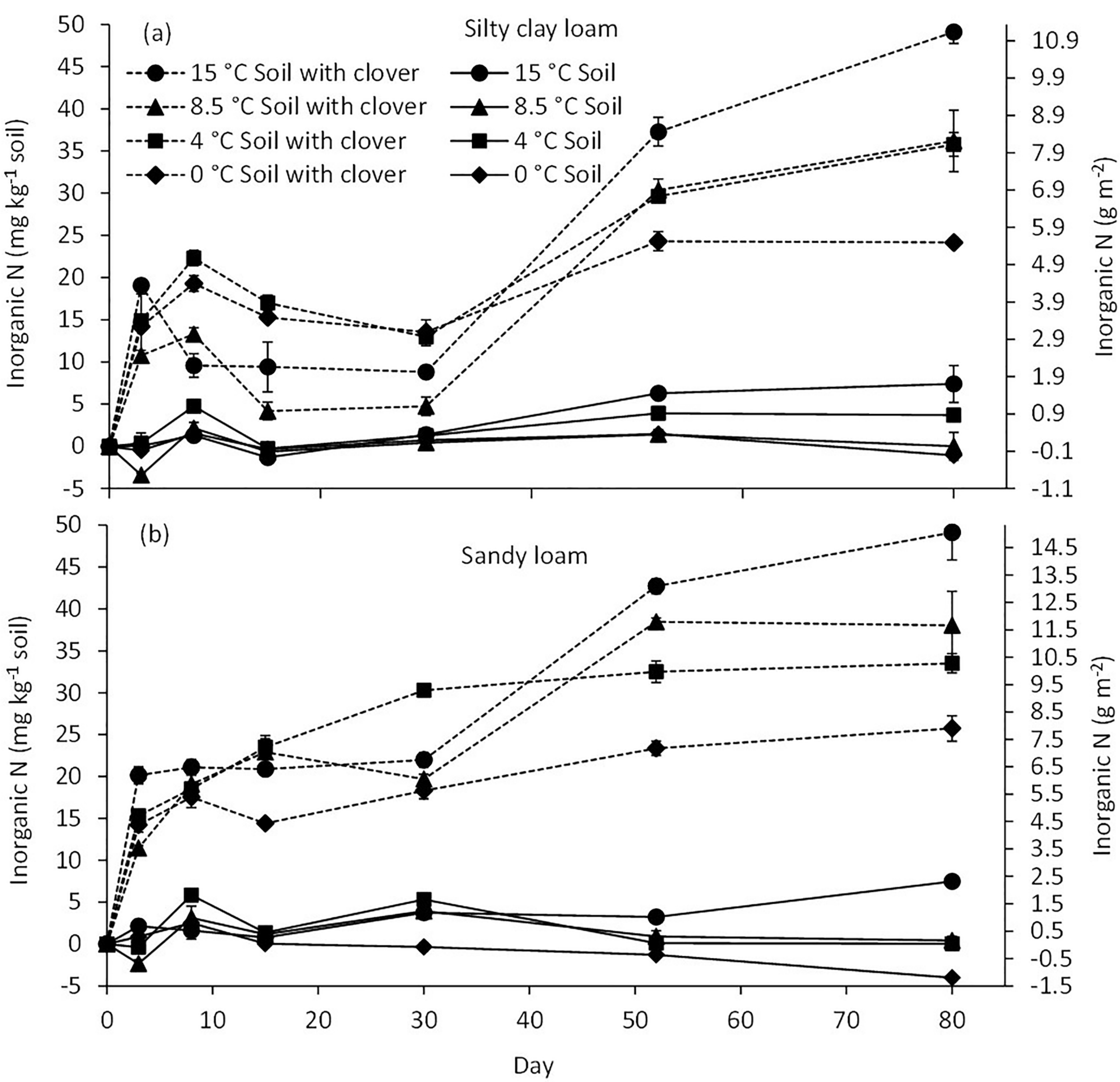

Fig. 1. Net inorganic N (ammonium-N + nitrate-N) during 80 days of incubation at 0, 4, 8.5 and 15 °C in (a) silty clay loam and (b) a sandy loam with clover leaves (dotted lines) and without (lines). Inorganic N at the start of the experiment is subtracted. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis shows the N content in a 20 cm soil layer assuming the soil original bulk density in situ (Table 1).

1982) on a Leco CHN 1000 analyzer (LECO Corp., St. Joseph, MI, USA). The results were expressed on a dry weight basis, as % of initial substrate and, for comparison with field data, converted to area units using appropriate bulk density values for 0–20 cm depth (Table 1). The net mineralized N was expressed as the change in inorganic N since the start of the experiment, i.e. the value at day 0 was subtracted. The net mineralized N from the clover leaves was calculated by the difference method: subtracting inorganic N in soil without clover leaves from inorganic N in soil with clover leaves. The mineralized C was calculated based on either the difference method or the measured fractional abundance of ^{13}C in the soils, in the ^{13}C-labelled clover leaves and in the CO_2 evolved in the chambers. More details about the calculations for estimating plant-derived and soil-derived CO_2 are given by Frøseth and Bleken (2015). For best visualisation of the relationship between N and C mineralization, we present the N/C ratio, rather than the C/N ratio.

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed using a general linear model (GLM) for soil inorganic N data and CO_2–C evolved as input data for each soil type and in total. Analyses were performed for all sampling occasions for each temperature and soil type using recordings for single chambers as input data. In advance, the dataset was checked for normality and homogeneity of variance. For multiple comparisons tests, Tukey HSD (honestly significant difference) procedure was used. A regression analysis was performed of net mineralized N vs. mineralized C on the two last sampling days, to test whether there was an effect of soil type on the N/C ratio of the mineralized products. The statistical software packages R (R Core Team, 2014) and SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA) were used for these calculations. In all tests, significance was assumed at P-levels ≤ 0.05.

3. Results

3.1. Net N mineralization from soil organic matter

The net mineralized soil N fluctuated during the first half of the incubation and most treatments showed an early peak on day 8 (Fig. 1). Only towards the end of the incubation, the net N mineralized, sum of ammonium and nitrate, showed a positive, though not thoroughly consistent, response to temperature (Fig. 1). The maximum amount was reached on the last incubation day at 15 °C. In quantity, it was nearly the same in both soils (7.4 and 7.5 mg N kg^{-1}soil). However, the decomposition rate at 15 °C, expressed as net mineralized N at day 80, in percentage of initial total soil N, was less than 1% (Table 2). In the silty clay loam (0.19%) it was less than a third of that in the sandy loam (0.68%).

After an initial period lasting up to 30 days, nitrate dominated the amount of the inorganic N, with highest levels in the silty clay loam (Fig. 2). For the most, ammonium was depleted, and more strongly at high than at low temperature (but there was no significant difference between the treatments at the end of the incubation) while nitrate increased and showed a significant positive response to temperature at the end of the incubation. At 0 °C the nitrate content in the sandy soil decreased (−3.2 mg kg^{-1} at day 80), suggesting immobilization.

3.2. Effect of added clover leaves on net N mineralization

Incorporation of clover leaves led to a rapid and non-temperature dependent increase in the content of inorganic N in both soils during the first three days of incubation (Fig. 1). Almost half of the total increase in the inorganic N content caused by decomposition of clover leaves was achieved within the first 8 days of incubation. This initial substantial increase in inorganic N was a real mineralization effect, as the amount of nitrate-N and ammonium-N released from a test with clover leaves not mixed with soil corresponded to only 2% of the inorganic N observed at day 3, or 0.1–0.2% of the clover N (data not shown).

Table 2
Net mineralized N (nitrate-N and ammonium-N) and C from clover leaves and soil organic matter as percent of initial N and C. Means ± standard errors are presented.

		N mineralized (% of initial clover N or soil N)				C mineralized (% of initial clover C or soil C)			
	Day	0 °C	4 °C	8.5 °C	15 °C	0 °C	4 °C	8.5 °C	15 °C
Clover leaves*									
Silty clay loam	3	7.7 ± 4.1	7.6 ± 0.7	7.5 ± 0.6	10.0 ± 0.4	2.3 ± 0.0	3.5 ± 0.1	5.3 ± 0.2	6.9 ± 0.3
	8	9.4 ± 1.0	9.2 ± 1.0	5.9 ± 0.8	4.3 ± 1.5	5.2 ± 0.1	8.3 ± 0.1	13.1 ± 0.5	14.9 ± 1.1
	15	8.2 ± 0.4	9.1 ± 1.0	2.5 ± 1.1	5.7 ± 2.7	8.4 ± 0.1	14.3 ± 0.0	17.4 ± 0.6	19.6 ± 0.7
	30	6.8 ± 1.5	6.1 ± 1.1	2.3 ± 1.1	3.9 ± 0.7	13.8 ± 0.3	19.1 ± 0.1	22.2 ± 0.4	23.2 ± 0.6
	52	12.0 ± 1.2	13.5 ± 0.6	15.2 ± 1.2	16.3 ± 1.8	17.2 ± 0.2	21.5 ± 0.2	24.7 ± 0.4	26.2 ± 0.6
	80	13.3 ± 0.5	16.9 ± 1.5	19.0 ± 3.8	21.9 ± 1.4	19.3 ± 0.2	23.3 ± 0.2	27.1 ± 0.5	28.5 ± 0.3
Sandy loam	3	7.0 ± 0.9	8.3 ± 0.7	7.3 ± 0.3	9.5 ± 1.0	1.0 ± 0.1	2.3 ± 0.3	6.1 ± 0.1	8.7 ± 0.6
	8	7.9 ± 1.3	6.7 ± 0.5	8.4 ± 1.8	10.2 ± 1.0	5.2 ± 0.2	9.9 ± 0.2	15.2 ± 0.4	18.0 ± 0.9
	15	7.6 ± 0.4	11.6 ± 1.5	11.4 ± 1.4	10.6 ± 0.5	9.5 ± 0.3	17.4 ± 0.2	22.5 ± 0.3	24.3 ± 0.9
	30	9.8 ± 1.0	13.1 ± 0.6	8.3 ± 0.6	9.6 ± 0.9	17.4 ± 0.3	23.4 ± 0.2	27.6 ± 0.3	28.3 ± 0.9
	52	13.0 ± 0.9	17.0 ± 1.4	19.8 ± 0.5	20.8 ± 0.9	21.4 ± 0.4	26.5 ± 0.1	30.8 ± 0.2	32.2 ± 0.9
	80	15.7 ± 1.6	17.6 ± 1.2	19.8 ± 3.7	21.9 ± 3.5	24.1 ± 0.4	28.5 ± 0.2	34.1 ± 0.2	35.8 ± 0.9
Soil**									
Silty clay loam	3	–0.012 ± 0.022	0.010 ± 0.062	–0.087 ± 0.012	0.001 ± 0.014	0.008 ± 0.001	0.009 ± 0.001	0.014 ± 0.002	0.025 ± 0.002
	8	0.039 ± 0.025	0.122 ± 0.015	0.056 ± 0.035	0.034 ± 0.026	0.014 ± 0.002	0.024 ± 0.001	0.035 ± 0.002	0.060 ± 0.006
	15	–0.008 ± 0.023	–0.007 ± 0.018	–0.016 ± 0.026	–0.034 ± 0.014	0.024 ± 0.002	0.042 ± 0.001	0.065 ± 0.003	0.110 ± 0.013
	30	0.018 ± 0.029	0.033 ± 0.015	0.011 ± 0.023	0.036 ± 0.028	0.041 ± 0.002	0.069 ± 0.003	0.115 ± 0.004	0.208 ± 0.027
	52	0.037 ± 0.009	0.101 ± 0.028	0.037 ± 0.028	0.162 ± 0.016	0.057 ± 0.003	0.099 ± 0.005	0.174 ± 0.005	0.312 ± 0.044
	80	–0.027 ± 0.033	0.095 ± 0.032	0.001 ± 0.083	0.190 ± 0.112	0.074 ± 0.002	0.138 ± 0.003	0.248 ± 0.005	0.433 ± 0.063
Sandy loam	3	0.080 ± 0.197	–0.033 ± 0.027	–0.212 ± 0.086	0.195 ± 0.136	0.023 ± 0.002	0.023 ± 0.002	0.036 ± 0.000	0.055 ± 0.000
	8	0.228 ± 0.053	0.532 ± 0.040	0.286 ± 0.249	0.146 ± 0.186	0.041 ± 0.003	0.051 ± 0.004	0.084 ± 0.003	0.128 ± 0.004
	15	0.004 ± 0.036	0.124 ± 0.070	0.105 ± 0.078	0.070 ± 0.051	0.059 ± 0.003	0.086 ± 0.002	0.139 ± 0.001	0.226 ± 0.002
	30	–0.030 ± 0.088	0.483 ± 0.052	0.358 ± 0.137	0.340 ± 0.105	0.089 ± 0.004	0.137 ± 0.002	0.215 ± 0.010	0.402 ± 0.002
	52	–0.115 ± 0.061	0.010 ± 0.068	0.082 ± 0.122	0.293 ± 0.034	0.115 ± 0.003	0.195 ± 0.002	0.315 ± 0.009	0.628 ± 0.004
	80	–0.365 ± 0.037	0.004 ± 0.131	0.040 ± 0.087	0.679 ± 0.067	0.152 ± 0.003	0.277 ± 0.006	0.476 ± 0.010	0.904 ± 0.005

*Clover leaves, for N: soil with clover minus soil without clover. For C: soil with clover minus soil without clover and minus priming effect. ** Soil without clover leaves, for N: differences from values measured on day = 0.

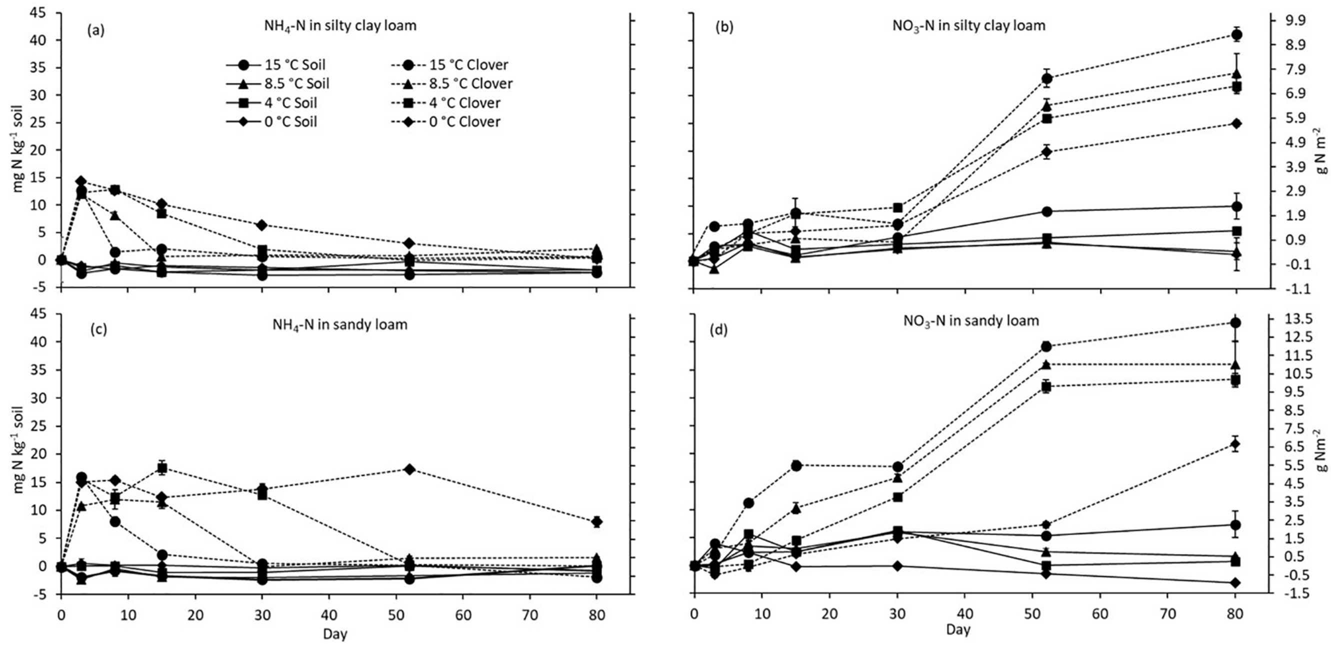

Fig. 2. Net ammonium-N and nitrate-N measured during 80 days of incubation at 0, 4, 8.5 and 15 °C in a silty clay loam (a and b) and a sandy loam (c and d) without clover leaves (lines) and as the additional effect of clover leaves added (dotted lines). Inorganic N at the start of the experiment is subtracted. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis as in Fig. 1. Both vertical axes are valid for each pair of plots (a-b and c-d).

Then, in the silty clay loam, a significant net immobilization occurred when incubated at the two highest temperatures (Fig. 1). In this period, the highest inorganic N content was found in soil incubated at the two lowest temperatures. In the sandy loam, there were similar tendencies, though the net immobilization was not significant; the inorganic N content was similar (day 14) or higher (day 30) when incubated at 4 °C compared to 8.5 °C or 15 °C. From day 52 onwards, the inorganic N content in both soils was highest in the highest temperature treatments. After 80 days of incubation, clover leaves increased the inorganic N content by 25–42 mg kg^{-1} in the silty clay loam and 30–42 mg kg^{-1} in the sandy loam. This corresponded to 13–22% of the clover N in the silty clay loam and 16–22% in the sandy loam (Table 2).

The rapid increase in inorganic N within the first days of incubation was mainly in the form of ammonium (Fig. 2). After this early peak, the amount of ammonium decreased and the nitrate level increased. The decrease in ammonium was faster with high than with low temperature, and faster in the silty clay loam than in the sandy loam (Fig. 2). At the end of the incubation, the enhanced ammonium level caused by incorporation of clover leaves had in most cases disappeared, and there was no significant difference between the temperature treatments in the soil ammonium content. An exception was found at 0 °C in the sandy loam, where the ammonium content was still substantially higher than at the higher temperatures.

3.3. C mineralization

In contrast to the rapid and unsteady changes in ammonium and nitrate, the net C mineralization remained rather constant and changed smoothly during the incubation. The rate of soil organic carbon (SOC) mineralization in the soil incubated without plant residues was strongly enhanced by temperature. It was about twice as fast in the sandy loam as in silty clay loam when expressed relative to the initial SOC (Table 2). At the end of the incubation, the total C loss was 0.074 to 0.43 % of the initial C in the silty clay loam, whereas in the sandy loam it was 0.15 to 0.90 %, at 0 °C and 15 °C respectively.

The rate of C mineralization of clover leaves was two order of magnitude larger than the mineralization rate of SOC (Table 2). It had a manifest bi-phasic trend, indicating that more readily decomposable substrates were gradually exhausted, most rapidly at higher temperatures, while less readily decomposable components of the clover leaves were mineralized at the end of the experiment (Fig. 3). The C mineralization of the clover leaves was also enhanced by temperature, but the effect was much smaller than for SOC. Depending on temperature, 19–29% and 24–36% of clover C was mineralized after 80 days of

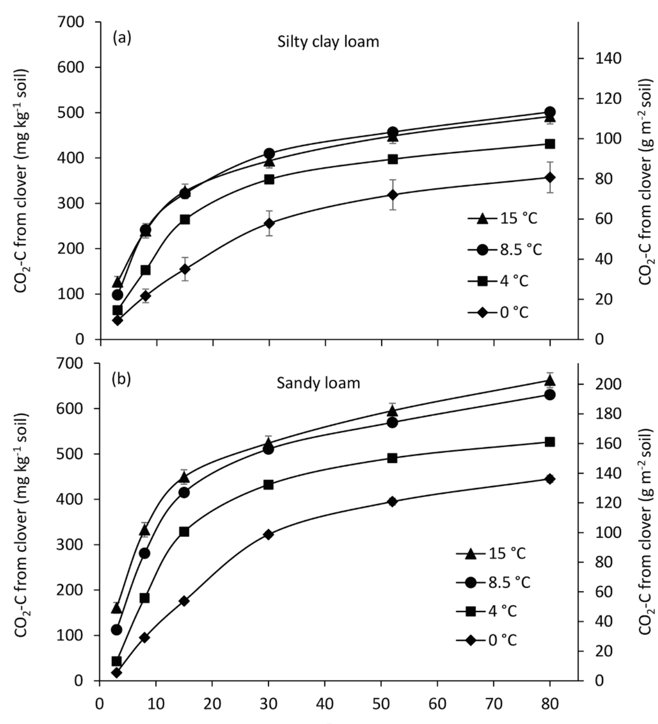

Fig. 3. Mineralized C from clover leaves during 80 days of incubation in (a) a silty clay loam and (b) a sandy loam, measured as accumulated CO_2-C per kg dry soil and per m^2 for 0–20 cm depth. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis as in Fig. 1.

incubation in the sandy loam and the silty clay loam, at 0 °C and 15 °C respectively (Table 2), indicating a significant effect of soil type on the decomposition of added plant litter. The incorporation of the clover leaves had also a positive priming effect on the decomposition of SOC (Frøseth and Bleken, 2015), which was conspicuous relative to the C mineralization of the soil alone, but quantitatively small relative to the amount of C evolved from the leaves.

3.4. N versus mineralized C

For the soil incubated alone, the net mineralized N was weakly related to cumulated mineralized C (data not shown). On the contrary, the plot of net mineralized N vs. cumulated C from clover, showed a very strong positive relationship, but only for the last incubation period, day 52 and day 80 (Fig. 4 and Fig. A1). During the first 30 days of incubation, the N immobilization phase, the relationship was negative in the silty clay loam and non-significant in the sandy loam.

The N/C ratio of net mineralized N to cumulated CO_2 emission from soil incubated alone showed a peak on the 8th incubation day, and was highest for the lower than for the higher temperatures (Fig. A2), whereas the N/C ratio of mineralized products from clover leaves showed clear time trends differentiated by temperature. In the first weeks of incubation, there was a markedly higher ratio of net mineralized N to mineralized C (Fig. 5). This high N relative to C mineralization was stronger at 0 °C and also at 4 °C than at higher temperatures, and stronger in the sandy loam than in the silty clay loam. Regarding the total mineralization products from both clover and SOC, the N/C ratio converged to 0.057 for the silty clay loam and 0.052 for the sandy loam (Fig. 5a and b). Based on the difference method, the N/C ratios of mineralized products from the clover leaves incubated at different temperatures converged to the same ratio, 0.054 for the clay and 0.052 for the sandy loam (Fig. 5c and d). When applying the ^{13}C-labelling method, instead of the difference method, on calculating the mineralized C from the clover leaves, the final N/C-ratio became higher; 0.075 for the silty clay loam and 0.063 for the sandy loam, with no significant difference (Fig. A3).

We explored whether the transition from a temperature-dependent N/C product ratio to a constant N/C ratio could be related to a change in the substrate, by plotting the N/C ratio of the mineralization products during each time interval against the cumulative amount of plant derived $^{13}CO_2$ (Fig. 6). After an initial plummeting, the N/C ratio showed a peak that for all treatment coincided in time on the second last interval (day 30 to day 52) but differed with respect to the amount of clover derived ^{13}C mineralized in advance, which was least at 0 °C and increased steadily with temperature. Depending on the temperature and soil, 14 to 28 % of the clover C was mineralized to CO_2 before the start of the peak period (Fig. 6). Including also CO_2 derived from priming of soil C in the x-axis, in addition to that derived from clover, did not modify the shape of the N/C trends (Fig. A4).

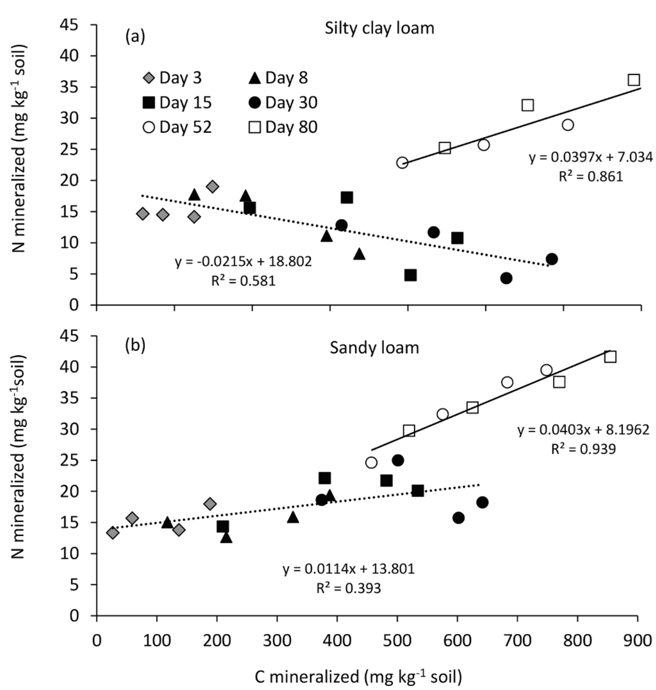

Fig. 4. Net mineralized N (ammonium-N + nitrate-N) vs. mineralized C from clover leaves during 80 days of incubation in (a) silty clay loam and (b) sandy loam. Both N and C were estimated by the difference method.

4. Discussion

4.1. Mineralization from soil alone

The preincubation of the soil should have been sufficient to remove the most of easily decomposable organic residues present in the soil. When studying the C release, we found that first order C mineralization rates could be easily identified (Frøseth and Bleken, 2015). The fluctuating pattern of mineralized N from soil alone at the beginning of the experiment can be partly a consequence of soil disturbance and also of experimental error, as indicated by the relatively large standard errors (Table 2). However, similar changes in the two soils, the consistent depletion of ammonium-N and the weak but statistically significant N immobilization in the soil incubated at 0 °C (about 1 g N/m^2 in the sandy soil), suggest that slow N turnover occurs also at temperatures close to the freezing point, as observed by Cookson et al. (2002) at 2 °C and Magid et al. (2001) at 3 °C. The observed significant temperature effect on net N mineralization in unamended soil is in line with several other studies (Cookson et al., 2002; Clark et al., 2009).

The importance of the choice of reference units can be illustrated by the fact that N mineralization from the two soil types in the absence of clover leaves was similar after 80 days of incubation when measured per kg soil, but when measured per g soil N it was more than three times higher in the sandy loam than in the silty clay loam. It follows that, when studying the effect of soil type on the turnover processes, relating the rate of mineralization to the substrate, i.e. to the amount of organic matter, is more informative than relating it to the mass of the bulk soil.

4.2. Decomposition of clover leaves

Since the net N mineralized from clover leaves was calculated by subtracting the N mineralization from soil alone, errors in the estimation of the latter will influence the results. However, since the amount of N mineralized from clover leaves was substantially more than N mineralized from soil, such errors have little influence on the results.

Our experiment was not designed to study the temperature sensitivity of nitrification. However, the results conform to results by e.g. Andersen and Jensen (2001) and Cookson et al. (2002), who found that nitrification is more sensitive to low temperature than is ammonification. Delayed nitrification at low temperatures can be positive for reducing the risk of N leaching, as ammonium is less prone to leaching.

Despite N-rich plant material, the total amount of N mineralized after 80 days of incubation at 15 °C was low (ca 22%) compared to that found in other studies. Cookson et al. (2002) found after 161 days of incubation at 15 °C that 60% of clover N had been mineralized, while 22% was mineralized when incubated at 2 °C, but they used 2.5 times as much clover residue per amount of soil as in our study. Van Schöll et al. (1997) found that 39% of rye shoot N was mineralized after 70 days of incubation at 15 °C. However, when we studied the soils under field conditions, the apparent N recovery in spring barley from mulched green manure incorporated in the spring was also low, i.e. around 7% (Frøseth et al., 2014). Since soil type affected the decomposition of plant residue, this can indicate that the contact between residue and soil can be important for the decomposition, in which case also the size of the

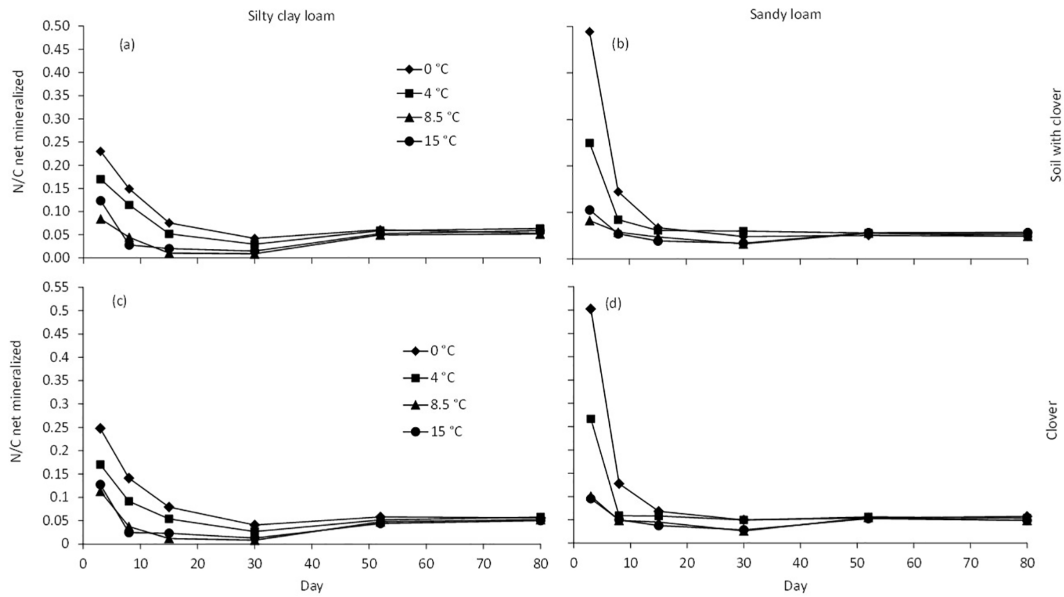

Fig. 5. The ratio of net mineralized N (ammonium-N + nitrate-N) to cumulated mineralized C from soil and clover (a and b) and clover (c and d) in silty clay loam and sandy loam during 80 days of incubation at different temperatures. Both N and C were estimated by the difference method.

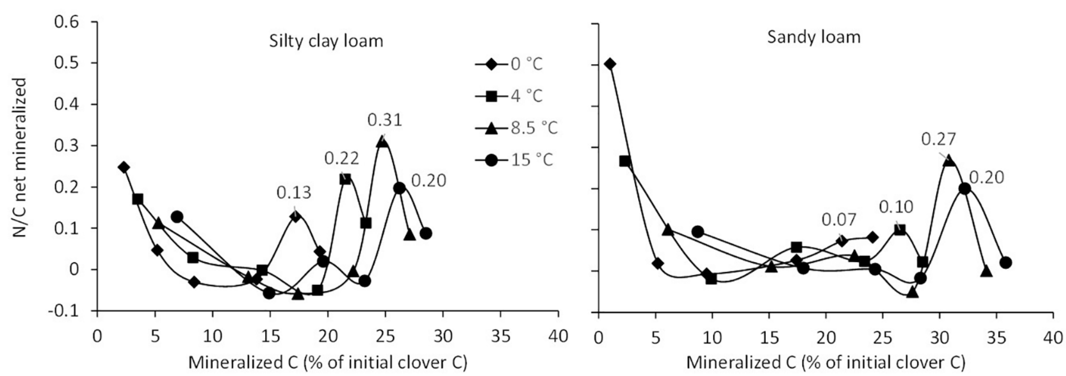

Fig. 6. The N/C ratio of mineralization products from clover leaves during each interval between two consecutive samplings (days 3, 8, 15, 30, 52 and 80) of incubation at different temperatures in two soils, calculated as the change in net mineral N divided by the C mineralized during the interval (estimated by the difference method, thus including priming of SOC). The N/C ratio is plotted against the net mineralized C from clover leaves only (by the ^{13}C method, thus excluding priming of SOC) as % of C in clover leaves.

residues would matter. In the field, plant residues from green manure are more heterogeneous, including larger plant parts as straw with a different biochemical composition and a higher C/N ratio than the leaves used in this study (Jensen et al. 2005). The low net N mineralization and N recovery may also indicate a longer turnover time of organic matter by soil microorganisms in the cold climate.

In line with our hypothesis (i), we found a rapid initial release of ammonium-N on the 3rd day after incorporation of the clover leaves, even at 0 °C. This corresponded to about 7% of the added N, and was independent of temperature in the tested range (0 to 15 °C). The rapid and considerable increase in net mineralized N due to the addition of clover leaves could also partly originate from an increased turnover of the soil organic matter (a real priming effect) or an increased turnover of the soil microbial biomass (an apparent priming effect) (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Labelling of the clover C in the present experiment showed a substantial priming effect (Frøseth and Bleken, 2015), but in lack of labelling clover N we could not quantify a priming effect on soil N mineralization. Since the C/N ratio of the bulk soils and of clover leaves was almost similar, close to 10–11, the stoichiometry of the mineralized C and N would not change much if all organic components were decomposed at a similar rate. If we estimate primed N based on the amount of primed C on the 3rd incubation day, the amount of primed N would be equivalent to only 4–6% of the mineralized N of the clover leaves on this day. Furthermore, the observed priming effect on soil C at the beginning of the incubation period was positively correlated with temperature, particularly in the sandy loam (on the 3rd day at 8.5 °C priming was 50% and 160% more than at 0 °C, in the clay and sandy loam respectively), while the early N mineralization (day 3) was not or little affected by temperature. Thus, priming effect alone does not provide a plausible explanation for the early N mineralization, and the rapid increase in mineral N seems to have been mainly caused by a substantial mineralization of the clover leaves. Clover leaves have a lower C/N ratio and contain more easily degradable compounds than the rest of the clover plant, such as stems and roots, which are commonly incorporated into the soil. Cookson et al. (2002) added clover material consisting of both shoots and roots and observed that the peak in ammonium-N, similar to that which we found after 3 days at all temperatures, in their experiment occurred later at lower temperatures (2 and 5 °C) than at higher temperatures (10 and 15 °C).

Also in line with our hypothesis (ii), the early ratio of net mineralized N to mineralized C from easily decomposable plant material was larger at lower than at higher temperature. An interpretation can be that the gross mineralization of plant material rich in easily degradable substances was affected less by low temperature than was microbial growth, as suggested by Magid et al. (2001) and confirmed by He et al. (2014). However, the observed conspicuous effect of temperature on the N/C ratio of mineralized products from clover decomposition gradually decreased and the N/C ratio converged at the two last sampling dates in both soils (Fig. 5), as the net N mineralized became a strong positive function of cumulated mineralized C, but not so up to day 30 (Fig. 4). After the initial mineralization peak (on day 3), along with a negative temperature effect on the N/C product ratio from clover leaves, there was a shift to net immobilization in some of the treatments. This immobilization was most pronounced in the silty clay loam, and it increased with higher temperatures. The observed shift between net mineralization to net immobilization at higher temperature is in line with several other studies, albeit we used plant material with lower C/N ratio than those, and it seems to take place between 3 and 5 °C and 8.5–11 °C (Andersen and Jensen, 2001; Magid et al., 2004; He et al., 2014). This may be explained by stronger enhancement with temperature of microbial growth (with a low C/N ratio) than of gross C decomposition, in line with the N/C ratio of the mineralized products, as mentioned. A net immobilization in the silty clay loam in the spring after ploughing the green manure ley may also explain the more severe early N deficiency and low barley yields on this soil compared to the same crop grown on the sandy loam, which we found under field conditions (Frøseth et al., 2014). Cookson et al. (2002) found comparable mineralization pattern when using constant temperatures (2, 5, 10 and 15 °C). Fluctuating temperatures occur in the field, and these may have different effects on N transformations than those of constant temperatures (Campbell and Biederbeck, 1972; Cookson et al., 2002). Cookson et al. (2002) found that immobilisation was the dominant process when the temperature decreased from 15 to 2 °C. The results indicate that N mineralization of plant material at low temperatures can supply plants with some N early in the season when the temperature is still low, in consistence with findings of He et al. (2014), but also that a period with immobilization may occur at somewhat higher temperatures, particularly in clay soils.

At low temperature, the decomposition of the substrate components is more separated over time than at higher temperatures (Magid et al., 2004). The duration of the initial first order mineralization period of clover C in our experiment depended on time and lasted between 8 and 30 days, longest at the lower temperatures, as expected (Frøseth and Bleken, 2015). In contrast, the net N to C mineralized ratio showed a peak during the second last interval regardless of temperature and regardless of the fact that the amount of clover ^{13}C already mineralized was least at 0 °C and increased steadily with temperature. The N/C product ratio at this peak interval was very high, highest at 8 °C and lowest at 0 °C (Fig. 6). At its most, it was 0.27–0.31 (C/N = [3.3, 3.7]) thus similar to that of pure proteins (N/C ≈ 0.3, C/N ≈ 3.3) and larger than what expected in bacteria (N/C) = [0.21, 0.25], C/N = [4.1, 4.8] (Mouginot et al., 2014). The N mineralization of clover leaves alone cannot support such a high N/C product ratio, and decomposition of bacteria and even N richer compounds as eso-enzymes and nucleotides, must be assumed, as well as a low microbial growth at this time interval. It should be noticed that this N/C peak in the net mineralized products did not seem to be connected to priming of SOC by the clover leaves, which was largest at the start and decreased gradually during the incubation (Frøseth and Bleken, 2015).

Our results support several other studies, which show that the effect of low temperature on organic matter turnover and mineralization is not simply that the same processes proceed at a reduced rate (Kirschbaum, 1995; Magid et al., 2001; Schütt et al., 2014). Thus, the data do not support the common modelling approach of an idealized rapidly decomposing pool with a specific N/C ratio as used in several models (Manzoni and Porporato, 2009). Different temperature regimes may affect the community composition of soil biology in long term (Wang et al., 2021), but temperature may also affect which organisms are active in the early phases of the decomposition process (Schmidt and Lipson, 2004). Furthermore, at temperatures close to 0 °C, the physiology of the microorganisms may change, for instance because they need to produce osmotica as frost protection, which altering their C to N relations (Schimel et al., 2007).

4.3. *Effect of soil type on N mineralization of clover leaves*

Net N mineralization was larger in the sandy loam than in the clay loam. This in agreement with Thomsen et al. (1996) and Soinne et al. (2021) who found that N-mineralization was negatively correlated with clay content. We expected that the ratio of net mineralization of N to mineralization of C from the clover leaves would not be affected by soil type. During the first weeks of incubation, contrary to our hypothesis (iii), we found that the N/C product ratio was higher in the sandy loam than in that of the silty clay loam, and that it was especially higher at the lower temperatures. This was so both when the difference method (which includes the primed soil C mineralization as plant mineralization) or the ^{13}C (which excluded primed soil C) were used. However, by the end of the incubation, the N/C ratios, based on cumulated C mineralization of clover leaves, including the priming effect on SOC mineralization, had converged to the same ratio in both soils and at all temperatures, in agreement with our hypothesis. Whether soil type influenced the N/C ratio product at the end of the experiment can be estimated also by the slope of N versus cumulative C mineralized. For the last two sampling dates this was about the same (≈ 0.04, Fig. 4), unaffected by soil type, as anticipated, but only if also the priming of SOC mineralization was included in the C mineralized due to clover leaves (see Fig. A1 for SOC priming excluded).

5. Conclusions

We studied the effect of low temperature and soil type on mineralized N and C from decomposing soil organic matter and N-rich plant material during 80 days of incubation. The results indicate that N mineralization of N-rich plant material proceeds at low temperatures, but also that a period with immobilization may occur in clay soils at somewhat higher temperatures. An initial rapid and substantial net N mineralization from the plant material took place in both soils at temperatures down to 0 °C. This was followed by a period lasting about 30 days during which net N immobilization was observed, stronger at higher than at lower temperature and stronger in the clay soil than in the sandy soil. At the end of the trial, a low amount (13–22%) of the total N in the clover leaves was mineralized.

The ratio of inorganic N to mineralized C during the first month of decomposition was higher at low temperatures than at high temperatures and was also influenced by soil type: higher in the sandy soil than in the clay soil. This shows that N mineralization is not simply a function of C mineralization in that phase, and that temperature as well as soil type affect the stoichiometry of the mineralization products. Later in the incubation, the C to N ratio converged to the same value for all temperature treatments in both soils. These results match field observations of early barley growth after spring incorporation of green manure, which presented more severe N deficiency in the clay than in the sandy soil at the start of the growing season. Thus, the effect of soil type on green manure mineralization should be included in planning for better N recovery, expecting a stronger immobilization in clay soils than in sandy soils after spring incorporation of plant residues and the soil temperature increases. While modelling is generally considered a practical way to estimate the N manuring effect of plant residues, these results present some challenges for the successful modelling of the stoichiometry of the mineralization products in the temperature range 0–15 °C, including the modifying effect of soil type on the mineralization of plant residues. A very large increase in mineralized N compared to mineralized C during the transition period from immobilization to net mineralization needs further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115483.

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