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Mineralization of carbon and nitrogen in soil samples taken from three fertilized pine stands: Long-term effects

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Abstract

Seven years after fertilization the rate of CO_2 production in the soil samples taken from the organic horizons of a poor pine forest site (*Calluna vulgaris* site type), treated with urea or ammonium nitrate with lime, was lower than that in the unfertilized soil. The same trend was also observed in samples of the *Empetrum-Calluna* site type 14 years after fertilization. In the more fertile *Vaccinium myrtillus* site type these rapidly-soluble N fertilizers had a long-term enhancing effect on the production of CO_2. Apatite and biotite eliminated the decreasing effect of urea on the production of CO_2. One reason for this might be the long-term increase in soil pH caused by apatite and biotite, or their constituents (Ca, Mg, K, P). Nitroform (a slow-releasing N fertilizer) had no statistically significant effect on the production of CO_2 in soil samples from any of the forest types. Despite the high N mineralization in the samples from nitroform fertilized soils there was no nitrification, and the high content of total N indicated that after nitroform fertilization the losses of N were low.

The correlation between the net mineralization values for C (CO_2 production) and N was poor. However, multiple linear regression analysis, which also took into account the effect of nutrients and pH, indicated that there was a link between the mineralization of C and N.

Introduction

Nowadays the nitrogen given in forest fertilization is in the form of rapidy-soluble compounds, generally urea or some nitrogen salt. Microbial activity is also affected by these fertilizers. Although nitrogen fertilization increases the number or biomass of microbes and microbial activity immediately after the addition (Foster *et al.*, 1980; Kelly and Henderson, 1978; Mai *et al.*, 1980; Roberge, 1976; Roberge and Knowles, 1967; Salonius, 1972; Söderström *et al.*, 1983), the number/biomass of microbes may decrease over time, even to a level below that in the unfertilized soil (Bååth *et al.*, 1981; Foster *et al.*, 1980; Popovic, 1977; Söderström *et al.*, 1983).

Slow-releasing fertilizers may play an increasing role in forest fertilization in the future. There are reports indicating that the stimulation of nitrification can be avoided, at least in some forest site types, by using slow-releasing nitrogen fertilizers (Martikainen, 1984). The lack of nitrification apparently reduces nitrogen losses and may increase the growth reaction of the tree stand to fertilization. However, the effects of slow-releasing fertilizers on mineralization processes in forest soil are poorly understood. The aim of the present work was to compare the long-term effects of fast and

Table 1. Soil chemical characteristics

	Treatment						Significance of the F-value[1]
	None	U	UAB	UABM	CaAN	NF	
MT site							
pH	4.23 (0.18)^{a}	4.37 (0.13)^{a}	4.65 (0.08)^{b}	4.60 (0.22)^{b}	4.59 (0.23)^{b}	4.28 (0.11)^{a}	***
EC $\mu S cm^{-1}$	103 (23)	100 (10)	113 (6)	113 (15)	123 (32)	110 (10)	
C %	36.7 (3.9)	35.8 (2.0)	33.2 (6.3)	30.8 (6.5)	34.4 (1.1)	38.7 (2.3)	
N %	1.15 (0.12)^{a}	1.26 (0.14)^{ab}	1.17 (0.09)^{a}	1.16 (0.09)^{a}	1.16 (0.09)^{a}	1.50 (0.23)^{b}	*
C/N	32.0 (1.7)	28.5 (2.9)	28.3 (3.2)	27.6 (3.7)	29.9 (3.1)	26.0 (3.1)	
Ca $\mu g cm^{-3}$	583 (126)^{ab}	583 (76)^{ab}	767 (67)^{b}	767 (208)^{b}	600 (50)^{ab}	442 (72)^{a}	*
K $\mu g cm^{-3}$	105 (18)	88 (10)	95 (36)	102 (3)	117 (34)	82 (36)	
Mg $\mu g cm^{-3}$	55 (13)^{a}	61 (14)^{a}	87 (15)^{a}	87 (25)^{a}	80 (18)^{a}	53 (6)^{a}	*
P $\mu g cm^{-3}$	13 (1)	12 (1)	16 (3)	13 (4)	14 (2)	12 (2)	
CT site							
pH	3.88 (0.13)^{a}	3.98 (0.04)^{ab}	4.35 (0.03)^{f}	4.47 (0.10)^{d}	3.98 (0.13)^{ab}	4.07 (0.16)^{b}	***
EC $\mu S cm^{-1}$	110 (20)	97 (15)	90 (10)	110 (26)	103 (12)	107 (12)	
C %	37.7 (6.9)^{a}	34.4 (0.3)^{a}	34.2 (2.1)^{a}	34.2 (2.2)^{a}	41.0 (2.2)^{a}	40.8 (1.0)^{a}	*
N %	1.10 (0.28)^{ab}	1.10 (0.06)^{ab}	1.03 (0.08)^{a}	1.10 (0.04)^{ab}	1.20 (0.06)^{ab}	1.41 (0.07)^{b}	*
C/N	34.7 (3.0)^{a}	31.4 (1.6)^{ab}	33.2 (0.9)^{a}	31.1 (0.3)^{ab}	34.0 (0.1)^{a}	28.9 (1.8)^{b}	**
Ca $\mu g cm^{-3}$	550 (100)^{a}	500 (50)^{a}	733 (58)^{a}	808 (101)^{b}	475 (25)^{a}	425 (25)^{a}	***
K $\mu g cm^{-3}$	92 (13)	85 (13)	83 (6)	102 (10)	78 (13)	71 (12)	
Mg $\mu g cm^{-3}$	35 (5)^{a}	45 (5)^{a}	85 (13)^{b}	97 (15)^{b}	53 (3)^{a}	38 (6)^{a}	***
P $\mu g cm^{-3}$	13 (0)^{ab}	10 (1)^{a}	13 (1)^{ab}	18 (6)^{b}	14 (2)^{ab}	12 (0)^{ab}	*
ECT site							
pH	3.75 (0.10)	3.66 (0.10)			3.77 (0.06)	3.73 (0.04)	
EC $\mu S cm^{-1}$	190 (8)	190 (16)			188 (29)	185 (21)	
C %	44.0 (4.2)	47.6 (1.0)			44.8 (2.7)	47.1 (2.7)	
N %	0.91 (0.15)	1.04 (0.05)			0.98 (0.09)	1.10 (0.03)	
C/N	49.0 (5.1)	45.8 (2.0)			46.1 (4.0)	43.0 (2.6)	
Ca $\mu g cm^{-3}$	406 (83)	381 (38)			400 (0)	325 (50)	
K $\mu g cm^{-3}$	213 (12)	195 (38)			190 (24)	188 (25)	
Mg $\mu g cm^{-3}$	64 (6)	64 (13)			75 (6)	60 (12)	
P $\mu g cm^{-3}$	20 (3)	17 (2)			17 (2)	17 (1)	

[1] *, **, *** significance at $P < 0.05$, < 0.01 and < 0.001, respectively. When the F-value was statistically significant, the means were tested. Those whose superscript has the same letter (rows) do not differ significantly ($P = 0.05$), standard deviation in parentheses.

slow-releasing nitrogen fertilizers on carbon and nitrogen mineralization in soils of three different site types under pine. The long-term effects of apatite, biotite and micronutrients on the mineralization of C and N in soils fertilized with nitrogen (urea) were also studied. Apatite and biotite are slow-releasing fertilizers (P, K, Mg) which also have a liming effect.

Materials and methods

The experimental sites and fertilization treatments

Two of the pine sites are located at Tammela in southern Finland. They were typed (according to Cajander, 1949) as *Vaccinium myrtillus* (MT) and *Calluna vulgaris* (CT). The third site is located at Kestilä in central Finland. It was typed as *Empetrum-Calluna* (ECT). The age of the stands was 65, 70 and 75 years, respectively. The soil pH and nutrient contents of the unfertilized organic horizon (O) are shown in Table 1. The thickness of the O horizon of the MT and CT sites was 2–7 cm and of the ECT site 4–8 cm. The average bulk density of samples taken from the O horizon (air-dry homogenized soil) of the MT, CT and ECT sites was 0.20, 0.22 and 0.25 $g cm^{-3}$, respectively.

Both of the sites at Tammela had six 10 × 10 m plots and twelve 40 × 40 m plots which were isolated by 5-m and 10-m-wide buffer strips, respectively. The fertilization treatments are shown in Table 2. The treatments had three replications (one 10 × 10 m and two 40 × 40 m plots). The fer-

Table 2. Fertilizers applied in the experiments on MT and CT sites at Tammela

Treatment	Abbreviation	Fertilizer, kg ha^{-1}	Nutrient, content mg ha^{-1} [a]
None			
Urea	U	432	N 200
Urea	UAB	432	N 200 P 44 K 83
Apatite[b]		203	
Biotite[c]		1563	
Urea	UABM	432	N 200 P 44 K 83
Apatite		203	B 1.1 Cu 12.8
Biotite		1563	Mn 5.5 Fe 9.8
Micronutrients		100	Zn 5.5 Mo 1.4 Na 0.7
Ammonium nitrate with lime[d]	CaAN	727	N 200
Nitroform[e]	NF	526	N 200

[a] Only total P and K from apatite and biotite, and N from CaAN are shown here. See the footnotes for the additional nutrients in these fertilizers.
[b] Particle size 40% < 53 μm, 90% < 210 μm, contains 14—15% P, 38% Ca, 0.9% Mg, 0.4% Al, 0.2% K and 0.1% Na.
[c] Particle size 90% between 210 and 1000 μm, contains 5.3% K, 1.0% P, 7% Ca, 5.0% Mg and 2.6% Fe.
[d] Contains 2.2% Mg and 4.0% Ca.
[e] Ureaformaldehyde, a slow-releasing nitrogen fertilizer (Hercules).

tilizer treatments were carried out in November 1978. The site at Kestilä had sixteen plots (20 × 20m) isolated by 10-m-wide buffer strips. The fertilizer treatments were laid out according to a Latin square design. There were four control plots and each nitrogen treatment (calcium ammonium nitrate, urea and nitroform) had four replicate plots. The amount of nitrogen was 150 kg ha^{-1}. Fertilization was done in May 1971.

Soil sampling and storage

The soil samples were taken at Tammela 7 years (June and August 1985) and at Kestilä 14 years (October 1985) after fertilization. At sampling 50 random samples (core diameter 10 cm) per plot were taken from the O horizon. After bulking, the samples were taken to the laboratory, homogenized by hand (all green material and roots thicker than about 1 mm were removed), and then stored in polythene bags at 4°C.

Soil analyses

Soil pH was measured in soil-water suspensions (1:2, v/v). Exchangeable NH_4^+ and combined NO_3^- + NO_2^- were determined in 2 *M* KCl extracts by alkaline steam distillation before and after addition of Devarda's alloy (Bremner, 1965). Total N was determined by the Kjeldahl method and C by the potassium dichromate method at Soil Analysis Service Ltd (Helsinki, Finland). Exchangeable Ca, K, Mg and soluble P were determined in 1.0 *M* ammonium acetate (pH 4.65) at Soil Analysis Service Ltd.

Laboratory experiments for the determination of CO_2 production and net mineralization of nitrogen

Twenty g of fresh soil were placed in six 600-ml flasks. The soil moisture was adjusted to 60% of the water-holding capacity. Exchangeable ammonium and combined nitrate and nitrite (see above) were determined from three of the replicate samples. Three flasks were stopped with rubber septa and incubated for 6 weeks at 14°C. The flasks were aerated periodically after determination of the concentration of CO_2 by gas chromatography (Martikainen, 1985a). After 6 weeks incubation the amount of exchangeable ammonium, nitrate and nitrite were determined as described above.

Table 3. The production of CO_2-C, net mineralization of N and the ratio of produced CO_2 to mineralized N

Parameter	Treatment							Significance of the F-value[1]
	Site	None	U	UAB	UABM	CaAN	NF	
mg CO_2-C cm^{-3} soil during 6 weeks at 14°C (I)	MT	1.30 (0.30)^{a}	1.46 (0.28)^{a}	1.69 (0.28)^{a}	1.82 (0.32)^{a}	1.58 (0.10)^{a}	1.32 (0.19)^{a}	***
	CT	1.35 (0.14)^{a}	1.13 (0.15)^{b}	1.53 (0.12)^{a}	1.95 (0.23)^{c}	1.19 (0.18)^{b}	1.42 (0.27)^{a}	***
	ECT	2.96 (0.40)	2.10 (0.41)			2.23 (0.45)	2.41 (0.28)	
Net mineralization of N ($\mu g\,cm^{-3}$ soil) during 6 weeks at 14°C (II)	MT	17.0 (9.9)^{a}	43.6 (15.0)^{a}	21.9 (15.2)^{a}	20.2 (9.5)^{a}	27.0 (8.8)^{a}	105 (26.2)^{b}	***
	CT	16.2 (8.6)^{a}	15.5 (10.0)^{a}	10.8 (7.6)^{a}	7.1 (12.9)^{a}	14.8 (4.8)^{a}	79.7 (31.7)^{b}	***
	ECT	3.7 (2.1)^{a}	2.0 (2.3)^{a}			5.4 (2.0)^{a}	16.6 (9.9)^{b}	*
Ratio of I to II	MT	110 (92)	35 (6)	112 (91)	106 (50)	54 (16)	13 (3)	
	CT	99 (50)	97 (64)	326 (394)	470 (519)	84 (22)	19 (3)	
	ECT	1044 (656)^{a}	613 (32)^{ab}			444 (121)^{ab}	234 (214)^{b}	*

[1] See Table 1 for the explanations.

Table 4. Coefficients of correlation between the net mineralization of carbon and nitrogen and soil characteristics

Parameter	Site	pH	C %	N %	C/N	Ca	K	P	Mg	Mineral-N^{a}	EC	CO_2-C produced during 6 weeks
CO_2-C produced during 6 weeks	MT	0.68 **	−0.79 ***	−0.54 *	−0.25	0.78 ***	0.39	−0.04	0.80 ***	0.27	0.41	
	CT	0.50 *	−0.36	−0.19	−0.14	0.63 **	0.59 **	0.74 ***	0.65 **	0.01	0.45	
	ECT	0.38	−0.45	−0.55 *	0.40	0.04	0.68 **	0.63 **	0.12	−0.16	0.44	
Net mineralization of N during 6 weeks	MT	−0.16	0.38	0.67 **	−0.37	−0.50 *	−0.22	−0.14	−0.31	0.62 **	0.10	−0.03
	CT	−0.26	0.49 *	0.69 **	−0.56 *	−0.57 *	−0.25	−0.09	−0.40	0.77 ***	0.24	0.07
	ECT	−0.02	−0.18	0.26	−0.46	−0.67 **	−0.32	−0.25	−0.53 *	−0.03	−0.15	−0.02

$^{a}\mu g\ (NH_4^+ + NO_3^- + NO_2^-)$-N cm^{-3} soil.
*, **, *** significance of r at $P < 0.05$, < 0.01, < 0.001, respectively.

Statistical analyses

The results were analyzed by correlation analysis, stepwise linear multiple regression, and analysis of variance. Comparison of the means after the analysis of variance was made by the Student-Neuman Keuls (SNK) procedure.

Results and discussion

The production of CO_2

At the CT site the production of CO_2 in the samples taken from plots treated with urea (U) and ammonium nitrate (CaAN) was lower than that in the samples from unfertilized soil. A similar trend was found also at the ECT site (Table 3). The results support the earlier observations that fertilization with nitrogen may decrease, some time after the treatment, the respiration rate in forest soil (Bååth *et al.*, 1981; Söderström *et al.*, 1983). However, the results presented in this paper suggest that the long-term effect depends on the site fertility and added N compound: a decreasing effect was found in the present work only in the case of the two poorer sites (CT and ECT) when the rapidly soluble compounds (U, CaAN) were applied (Table 3). The slow-releasing N fertilizer (NF) had no statistically significant effect on the production of CO_2 (Table 3).

The long-term increasing effect of apatite and biotite on soil pH (Table 1) might be one reason why they stimulated CO_2 production and counteracted the decreasing effect of urea (Table 3); the CO_2 production correlated positively with the soil pH in samples from both the MT and CT sites (Table 4). Liming is known to have a long-term increasing effect on the production of CO_2 in forest soil (Lohm *et al.*, 1984; Williams, 1972). The correlations between Ca, Mg and the CO_2 production in the samples from the MT and CT sites were positive (Table 4). Addition of Ca and Mg has been found to increase respiration in forest soil fertilized with urea (Roberge, 1976). In the present work there was a positive correlation between pH and Ca in the samples from both the MT and CT sites ($R = 0.65^*$ and 0.72^*), as well as between pH and Mg ($R = 0.90^{***}$ and 0.85^{***}). Although stepwise linear multiple regression did not select pH for the models (Table 5), the effect of pH is also included in the regression coefficients for Ca (CT site) and Mg (MT site).

The CO_2 production in the samples from the CT site correlated positively, as was the case for the ECT site, with P and K (Table 4). These nutrients did not correlate strongly with pH in the samples from any of the sites. Especially in the case of fertilized soil from poor sites, P and/or K might increase the decomposition activity as the correlation (Table 4) and regression analysis (Table 5)

Table 5. Regression models for the production of CO_2-C (mg C cm^{-3} soil during 6 weeks in the laboratory at 14°C, n = 18 for the MT and CT sites, and 16 for the ECT site)

Variable	Regression coefficient	P	Partial correlation	Constant	R^2
MT site					
Mg	0.010	0.0002	0.783		
Mineral-N[a]	0.018	0.0070	0.628		
N %	−0.614	0.0206	−0.495		
				1.227	0.803
CT site					
P[a]	0.060	0.0001	0.720		
Ca[a]	0.00099	0.0011	0.602		
				0.062	0.784
ECT site					
C %	−0.078	0.0057	−0.696		
K[a]	0.0092	0.0174	0.623		
P[a]	0.084	0.0444	0.544		
				2.719	0.767

[a] $\mu g\,cm^{-3}$ soil.

suggested. Phosphorus (Hendrickson, 1985; Williams, 1972) and K (Roberge, 1976) have been found to enhance decomposition in forest soil.

The highest CO_2 production in the MT and CT sites occurred in samples from plots treated with urea + apatite + biotite + micronutrients (Table 3). However, it was difficult to evaluate the nutrient effect of added micronutrients on the decomposition rate because this application damaged the moss cover (unpubl. results). The extra carbon released from the damaged mosses might enhance the production of CO_2.

The incubation experiments using soil from the small (10 × 10 m) plots of the MT and CT sites were repeated in August 1985. The relative differences between various treatments in the CO_2 evolution (results not shown) were quite similar to those reported above for the samples taken in June 1985.

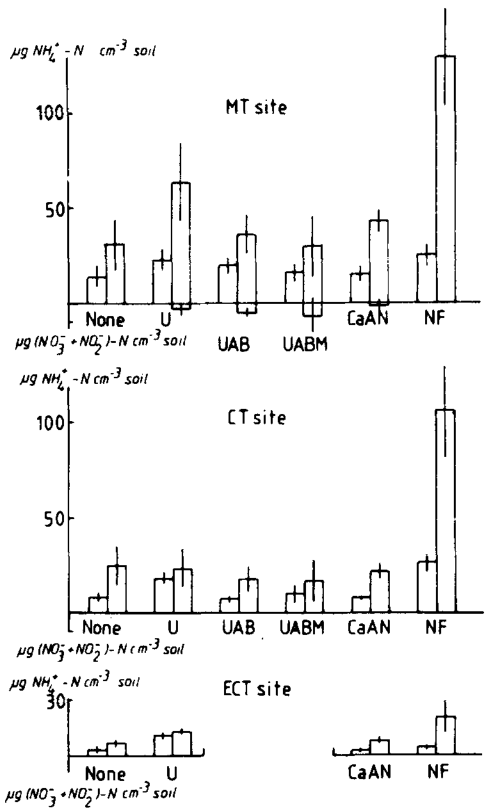

Fig. 1. NH_4^+-N and (NO_3^- + NO_2^-)-N in the organic horizon of the MT, CT and ECT sites before soil incubation (*the first column of each treatment*) and after 6 weeks at 14°C (*the second column of each treatment*). Mean of 3 replicate plots (3 replicate incubations per plot). Standard deviation is shown by *bars*.

Net mineralization of nitrogen

The net mineralization of N in the samples of the MT and CT sites taken in June 1985 (Table 3) and in August 1985 (results not shown) was the highest in the soil fertilized with nitroform. In the case of the samples from the ECT site the highest net mineralization activity was found in the samples from soil treated with nitroform (Table 3). Net mineralization of N in the samples from plots treated with the other fertilizers did not differ statistically significantly from the unfertilized plots (Table 3). In contrast to urea, nitroform did not stimulate nitrification (Fig. 1). This is in agreement with earlier observations (Martikainen, 1984; 1985b). The results indicated that nitrogen losses from soil fertilized with nitroform are low; it was rather surprising that the increasing effect of nitroform on the total N content could still be detected 7 years after the treatment (Table 1). In the samples from the MT and CT soils treated with nitroform the ratio of CO_2-C produced to accumulated N was low (Table 3). The low ratio indicated that a substrate with a low C to N ratio can be decomposed. This substrate might be undecomposed nitroform.

Apatite and biotite had (Fig. 1), as earlier demonstrated (Martikainen, 1984), an increasing effect on nitrification in the MT soil treated with urea. Their effect on the net nitrification resembles that reported for limestone (Carey *et al.*, 1981; Nömmik, 1978, 1979), indicating that the long-term increase in soil pH which they caused stimulated nitrification activity.

Ca and Mg correlated negatively with mineralization of N but positively with mineralization of C. Similar trends were also found for the effects of P, K and soil pH (Tables 4, 5 and 6). Many studies have shown that the relationship between C and N mineralization in forest soil is complex. Manipulation of the soil will increase the net mineralization of C (the production of CO_2), but decrease the net mineralization of N, or *vice versa* (Hendrickson, 1985; Lohm *et al.*, 1984; Nömmik, 1978; Popovic, 1984; Zöttl, 1960). For example, raising the pH of acid humus will, in general, increase the production of CO_2 but decrease the net mineralization of N since it supports the immobilization of N. The results obtained in the present study agree with this generalization. Although there was no significant correlation

Table 6. Regression models for the net mineralization of N (μg N cm^{-3} soil during 6 weeks in the laboratory at 14°C, n = 18 for the MT and CT sites, and 16 for the ECT site)

Variable	Regression coefficient	P	Partial correlation	Constant	R^2
MT site					
N %	161.0	0.0000	0.854		
CO_2-C[a]	107.6	0.0002	0.789		
Mg[b]	−1.204	0.0023	−0.686		
				−237.4	0.792
CT site					
CO_2-C[a]	103.9	0.0000	0.862		
Ca[b]	−0.172	0.0000	−0.828		
P[b]	−5.805	0.0003	−0.753		
N %	53.36	0.0237	0.587		
				−10.61	0.890
ECT site					
Ca[b]	−0.085	0.0025	−0.719		
C/N	−0.803	0.0306	−0.558		
				75.96	0.619

[a] mg cm^{-3} soil during 6 weeks at 14°C.
[b] μg cm^{-3} soil.

between the mineralization of C and N (Table 4), the link between these two processes became apparent (CT and MT sites) in stepwise linear multiple regression (Table 6). This is quite natural since the nutrients and pH had a counteracting effect on these processes, and the effect of C mineralization on the mineralization of N cannot be seen without taking into account the effect of nutrients and pH. Thus an increase in the mineralization of C apparently supported the mineralization of N (gross mineralization). We can conclude that apatite and biotite also increase the mineralization of N, in addition to the mineralization of C in the humus from the MT and CT sites, although the net mineralization of N in the incubation experiments was decreased by apatite and biotite (Table 3).

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