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REGULAR ARTICLE

Comparison of indices for the prediction of nitrogen mineralization after destruction of managed grassland

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Abstract Intensively managed grasslands are occasionally chemically killed with herbicide and ploughed in order to grow an arable crop. After this management, large N mineralization rates with large losses to the environment are commonly observed. However, it remains to be determined to what extent the chemical killing contribute to increased N mineralization. In this study the potential nitrogen (N) mineralization from grasslands, that were killed with herbicides but otherwise undisturbed, was investigated in a laboratory experiment with undisturbed soil columns. Subsequently we assessed the predictive value of several laboratory indices for N mineralization after chemically killing of the grass. Mineralization rates varied from 0.5 to 3.0 g N m^{-2} wk^{-1}. The contents of total N, total C, dissolved organic carbon (DOC) and hot-KCl extractable NH_4^+ were best related to N mineralization rates (R^2=50, 48, 38 and 47%, respectively). In combination with information on the N content of the roots and stubble and the age of grassland at destruction, up to 62% of the variation in N mineralization rates could be explained. Although previous studies suggested that dissolved organic nitrogen (DON) is a good indicator for mineralization rates, this was not the case after chemically killing grass in the current study.

Keywords Mineralization rates · Laboratory indices · Total N · Dissolved organic carbon (DOC) · Total C · Dissolved organic nitrogen (DON) · Hot-KCl extractable NH_4^+

Introduction

European intensive dairy production systems are often based on rotations of grasslands and arable crops (Taube and Conijn 2004). In these systems, grasslands are occasionally treated with herbicides and ploughed in order to grow an arable crop or for reseeding with high yielding grass varieties. As result of grassland destruction, the content of mineral N in the soil strongly increases (up to more than 200 kg N ha^{-1}) (Besnard et al. 2007; Bommelé 2007; Davies et al. 2001; Eriksen and Jensen 2001; Johnston et al. 1994; Nevens and Reheul 2002). This increase in mineral N content is caused by a combination of mineralization of the grass biomass (stubbles and roots) and by enhanced mineralization of organic N that accumulated under the forage grass. When periods with high soil mineral N contents coincide with wet conditions, the risk on NO_3^- leaching, denitrification losses and the emission of the greenhouse gas nitrous oxide (N_2O) is high (Adams and Jan 1999; Ball et al. 2007; Hansen et al. 2007; Lloyd 1992; Shepherd et al. 2001).

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As ploughing generally has a strong effect on the soil mineral N content through mineralization of soil organic matter, chemically killing of grassland without ploughing may be a strategy to decrease the risk of N losses. Fine-tuning the rates and timing of N fertilizer applications to the following crop, taking into account predicted N mineralization of the killed sward, might help to reduce N losses. For example, Aarts et al. (2001) and Nevens and Reheul (2002) showed that N fertilizer can be withheld without yield loss for maize in the first year after grassland ploughing. However, this requires quantitative insight in the N mineralization rates after killing the sward and the availability of N in the soil, both directly after the sward has been killed and later on.

There are several options to gain this insight in mineralization rates. Published studies on predicting plant available N in the soil focus either on biological (incubation) or chemical (extraction) laboratory indices (Keeney 1982), on models (Bhogal et al. 2001; Thornley and Verberne 1989), or on field methods (Bhogal et al. 1999; Hatch et al. 2000; Wienhold 2007; Besnard et al. 2007). Field methods have the advantage that they may determine the N mineralization rates under realistic conditions, but their results may be difficult to generalize. Moreover, these methods are much more time consuming than laboratory indices that can be implemented as routine analyses in laboratories. Published laboratory indicators for N mineralization rates in permanent arable and grassland soils include total N and C contents (Accoe et al. 2004; Hassink 1995), hot KCl extractable NH_4^+ (Gianello and Bremner 1986), and soluble organic N (DON) (Groot and Houba 1995). DON has shown to be a promising indicator in other studies (Murphy et al. 2000). However, these studies used mixed soil material. To study the effects of chemically killing the sward, the soil should not be disturbed or mixed. However, it is unknown to what extent the known methods predict N mineralization rates in undisturbed soils containing large quantities of plant residues. Therefore the predictive value of the indices has to be determined in this study.

The main objective of our study is to quantify the potential N mineralization rates in grasslands on a range of grassland soils that were chemically killed with herbicide but otherwise undisturbed. As fine-tuning N fertilizing in the subsequent crop to N mineralization rates may contribute to reducing N losses, our second aim is to assess a series of laboratory indices as predictors of N mineralization rates in the same set of soils.

Materials and methods

Field selection and sampling

In the Netherlands, grassland renewal occurs on both clay soils and sandy soils and includes grasslands of different ages (Schils et al. 2002). Grasslands on sandy soils are on average ploughed every 5 years and grasslands on clay soils every 10 years. A total of 42 grassland fields, varying in soil organic matter content and grassland age, were selected within 13 dairy farms where management records were accurate and easily available. Seven farms were located on sandy (to sandy loam) soils and the other six on clayey (silt loam to heavy clay) soils. Grassland ages were recorded, except for a few old grassland fields (>20 years old) where the exact age was not known and therefore estimated. For statistical analysis, grassland ages for the old fields were set to 30 years. The texture of the soils was estimated based on the guidelines for soil description (FAO-ISRIC 1990).

As our study was on chemically killed, but otherwise undisturbed swards, our experimental set-up differed markedly from most of previous studies quantifying mineralization rates in sieved soil after removal of fresh crop residues. In each field, two undisturbed soil columns of 19.5 cm diameter and 15.0 cm height (including the grass/sward that remained after cutting) were sampled within 15 cm from each other. Sampling was performed immediately after the first grass cut in April and before application of fertilizers. Destruction of grasslands in the Netherlands generally takes place in this period. Six of the farms were sampled in May 2005, the rest in May 2006. In both years, an identical sampling procedure was followed.

Experimental set-up

One of the two soil columns per field was used for soil and plant analyses and was vertically divided in two halves. This was done within one or 2 days after sampling. One half, used for measuring soil parameters at the start of the experiment, was dried at 40°C, weighed and ground after biomass (stubbles and roots) was removed from soil by sieving over a 2 mm sieve. The other half, used for measuring and analyzing plant biomass, was rinsed with water in order to remove all soil particles from grass stubbles and roots. The biomass was dried at 70°C, weighed and ground.

The remaining column for each field was stored in a 6-liter Mitscherlich pot for determination of net N mineralization in undisturbed soil. In all columns, moisture contents were kept below field capacity during the incubation (gravimetric soil moisture 0.20–0.40 g g^{-1} corresponding to water-filled pore space (WFPS) of 24–29% and 29–35% for the sandy and clayey soils, respectively). Samples were gravimetrically adjusted to a soil moisture content of 0.20 g g^{-1}.

The grass in the columns was chemically killed using glyphosate, which is the common chemical used for grassland destruction in the Netherlands. The columns were kept at 15°C for 2 weeks and were exposed to light during this period, in order to facilitate the initial enhanced growth of grass after glyphosate that is usually observed. After this period of 2 weeks, when the grass was dead, three small soil cores (1 cm diameter and 15 cm length) were sampled and combined. These soil samples were analysed for contents of NO_3^-, NH_4^+, DON and DOC, using the methods as described below. The columns were subsequently incubated at 20°C for 12 weeks. Sampling and soil analysis was repeated after 2, 6 and 12 weeks, using the same procedure of taking small cores. The holes were filled with plastic tubes to prevent the soil from drying.

Laboratory analyses on indicators in soil and biomass

Several indicators were quantified on all soil samples. Total N contents were measured spectrophotometrically after digestion with a mixture of sulphuric acid, salicylic acid, Se and H_2O_2; total C contents were determined spectrophotometrically according to Kurmies (Houba et al. 1997; Temminghoff 2000). The contents of NH_4^+, NO_3^+, soluble organic C (DOC) and soluble organic N (DON) in soil were analysed after extraction in a 1:10 (v/v) ratio with 0.01 *M* $CaCl_2$ (Houba et al. 2000). The NO_3^- and NH_4^+ concentrations were determined using standard segmented-flow analysis. Total content of DON was calculated as the difference between total dissolved N and mineral N (i.e. NH_4^+-N+NO_3^--N). The release of NH_4 during boiling of soil at 100°C in KCl has shown to be a promising indicator for N mineralization rates (Curtin et al. 2006; Gianello and Bremner 1986; Smith and Li 1993). Hot KCl extraction was carried out on soil and biomass through a 4 h extraction of 6 g air dry soil with 40 ml 2 *M* KCl in 100 ml incubation bottles placed in a water bath of 100°C The bottles were regularly stirred during the extraction. The extraction solution was filtered over a 5-µm filter and analysed for NO_3^- and NH_4^+ using standard segmented-flow analysis techniques. The hot KCl extractable NH_4^+ was calculated as the difference between NH_4 after boiling with 2 *M* KCl and the NH_4^+ content derived from the 0.01 *M* $CaCl_2$ extraction data.

Data analyses

A t-test showed no significant differences in N mineralization rates between the samples of both years. All samples were therefore treated as one population and jointly analyzed. To determine the net N mineralization rate during the 12 week incubation period, a linear regression line was fitted through the mineral N after 0, 2, 6, and 12 weeks. Data analyses were both performed per soil type and on all data combined. Correlations among soil parameters were determined by Pearson's correlation analyses. In order to find an indicator that predicts total net mineralization after 12 weeks, single and multiple linear regression analyses were performed with all measured soil and biomass parameters (total C, total N, DOC, DON, Hot-KCl extractable NH_4 in soil and total N in crop). The age of grassland and soil type were also included in the multiple regression analysis,. All statistical analyses were carried out with SPSS 15.0.

Results

N mineralization rates

The mineral N contents during the 12 weeks of incubation could be modelled using linear regression (Table 1). Mineralization rates varied from 0.5 to 3.0 g N m^{-2} $week^{-1}$. Mineralization rates did not differ significantly (p=0.066) between sandy soils (mean 1.2 ± standard deviation of 0.5 g N m^{-2} wk^{-1})

Table 1 Initial soil and crop properties (texture classes: 1=sand; 2=loamy sand; 3=silt loam; 4=clay loam; 5=clay; 6=heavy clay). The regression model (Y=aX+b), where Y is the amount of mineral N (g m^{-2}), X the time (weeks), b the amount mineral N (g m^{-2}) at start of the incubation and a the mineralization rate (g m^{-2} wk^{-1})

nr	Location	Field	Sampling year	Soil type	Bulkd.	Texture class	Age years	Regression model	R^2	Biomass g (d.m.) m^{-2}
1	Gouthum	B3	2006	Clay	0.95	4	6	y=1.600 x+3.014	0.99	828
2	Gouthum	B3	2006	Clay	0.87	5	6	y=1.221 x+3.330	0.98	666
3	Gouthum	B4	2006	Clay	0.81	5	>30	y=2.019 x+7.141	1.00	654
4	Gouthum	C2	2006	Clay	1.05	4	4	y=1.601 x+5.983	0.99	851
5	Gouthum	C4	2006	Clay	0.75	4	>30	y=2.020 x+3.419	0.99	799
6	Gouthum	E5	2006	Clay	0.82	5	>30	y=2.514 x+5.114	1.00	759
7	Zeewolde	4	2005	Clay	1.32	4	7	y=1.022 x+5.784	1.00	1,110
8	Zeewolde	8	2005	Clay	1.35	4	3	y=0.732 x+3.201	1.00	754
9	Westergeest	12	2006	Clay	0.88	5	1	y=1.502 x+3.196	1.00	526
10	Westergeest	15	2006	Clay	0.74	5	>30	y=1.986 x+3.292	1.00	742
11	Westergeest	18	2006	Clay	1.06	5	2	y=1.118 x+2.312	0.99	714
12	Westergeest	19	2006	Clay	0.79	5	>30	y=3.070 x+6.378	1.00	588
13	Bedum	J4	2005	Clay	1.03	5	8	y=1.972 x+4.228	1.00	574
14	Bedum	O11	2005	Clay	0.99	5	13	y=1.438 x+1.289	0.99	911
15	Waardenburg	8	2005	Clay	0.96	6	8	y=1.649 x−0.762	0.91	1,434
16	Waardenburg	10	2005	Clay	1.08	6	8	y=2.208 x−1.251	0.94	849
17	Lelystad	117-5	2006	Clay	1.33	3	5	y=0.838 x+2.752	0.99	524
18	Lelystad	117-5	2006	Clay	1.50	3	2	y=0.982 x+5.693	0.83	722
19	Lelystad	J58-02	2006	Clay	1.30	4	2	y=0.800 x+5.272	0.98	515
20	Lelystad	J58-03	2006	Clay	1.38	4	1	y=0.691 x+5.596	0.95	153
21	Lelystad	J59-01	2006	Clay	1.21	4	20	y=0.953 x+4.652	0.97	631
22	Lelystad	J59-06	2006	Clay	1.40	4	3	y=1.462 x+11.440	0.90	208
23	Heino	1	2006	Sand	1.44	2	7	y=1.775 x+2.037	0.99	629
24	Heino	22–25	2006	Sand	1.22	2	8	y=1.291 x+4.196	0.92	1,062
25	Heino	47	2006	Sand	1.32	2	2	y=1.053 x+4.636	0.92	488
26	Heino	8-12-1	2006	Sand	1.37	2	1	y=0.494 x+1.048	1.00	298
27	Heino	8-12-2	2006	Sand	1.38	2	1	y=0.594 x+1.505	0.98	454
28	Heino	8-12-3	2006	Sand	1.41	2	3	y=1.168 x+1.824	1.00	711
29	Soerendonk	1	2005	Sand	1.39	1	13	y=1.679 x+8.657	0.96	759
30	Soerendonk	3	2005	Sand	1.29	1	13	y=1.145 x+10.831	0.87	685
31	Soerendonk	7	2005	Sand	1.38	2	13	y=0.907 x+1.307	1.00	1,393
32	Landhorst	2	2005	Sand	1.33	1	7	y=2.115 x+7.791	0.90	849
33	Landhorst	6	2005	Sand	1.33	1	4	y=1.276 x+5.912	0.99	450
34	Hengelo	8	2005	Sand	1.35	2	5	y=0.803 x+2.751	1.00	995
35	Hengelo	17_2	2005	Sand	1.42	1	16	y=0.625 x+2.635	1.00	744
36	Hengelo	K1	2005	Sand	1.58	2	2	y=1.039 x+3.030	0.97	873
37	Ysselsteyn	2	2005	Sand	1.00	1	11	y=1.685 x−0.321	0.79	1,264
38	Ysselsteyn	12	2005	Sand	1.28	2	4	y=1.804 x+1.913	0.99	696
39	Maarheeze	1	2005	Sand	1.42	2	7	y=1.304 x+10.049	0.54	752
40	Maarheeze	12	2005	Sand	1.24	2	9	y=1.506 x+9.180	0.92	850
41	Voorthuizen	bos	2006	Sand	1.37	1	5	y=0.633 x+2.626	0.92	750
42	Voorthuizen	huis	2006	Sand	1.34	1	10	y=0.906 x+11.488	0.89	218
Mean								Rate=1.36 (g Nm^{-2} wk^{-1})		725
SD								0.57 (g Nm^{-2} wk^{-1})		277

Table 1 (continued)

nr	Biomass N g m^{-2}	Total C g kg^{-1}	Total N g kg^{-1}	C/N	NO3-N mg kg^{-1}	NH4-N mg kg^{-1}	Nmin mg kg^{-1}	DOC mg kg^{-1}	DON mg kg^{-1}	DOC/DON	Hot-KCL mg NH4^{+}-N kg^{-1}
1	12.2	55.8	5.4	10	13	6	19	615	41	15	54
2	10.5	54.0	5.3	10	13	13	25	818	74	11	49
3	10.8	68.6	7.0	10	33	14	47	777	65	12	78
4	15.1	45.3	4.4	10	17	9	26	492	42	12	45
5	10.9	67.1	7.0	10	13	7	20	715	49	15	73
6	11.2	69.8	7.0	10	18	8	26	824	52	16	74
7	14.0	32.1	2.4	13	5	4	9	185	16	11	25
8	11.8	28.5	2.1	13	4	3	7	162	14	12	22
9	9.0	68.9	6.4	11	20	9	29	829	53	16	72
10	13.8	69.4	6.7	10	18	9	27	850	60	14	70
11	9.5	50.2	3.7	14	2	6	9	423	26	16	46
12	12.9	69.7	6.9	10	36	13	49	714	59	12	74
13	10.5	49.2	4.6	11	5	4	9	434	28	15	42
14	12.3	36.2	3.2	11	3	4	7	281	18	16	29
15	18.0	40.6	4.1	10	9	8	16	480	26	18	35
16	10.6	45.5	4.3	11	5	4	9	550	30	18	38
17	9.5	27.5	2.2	12	10	7	17	278	25	11	26
18	11.0	22.5	1.7	13	13	4	17	174	18	10	20
19	10.2	25.5	2.0	13	13	6	19	208	21	10	22
20	3.8	24.2	1.8	13	24	16	40	141	14	10	21
21	10.7	29.6	2.4	12	4	2	5	225	23	10	29
22	5.0	27.9	2.4	12	4	2	6	318	37	9	24
23	10.7	30.2	1.6	20	4	15	19	139	15	9	23
24	16.7	26.1	2.3	12	5	11	15	200	19	10	29
25	8.3	26.8	1.9	14	4	7	11	125	13	10	25
26	3.7	30.8	1.4	21	1	2	3	75	7	10	18
27	5.2	28.5	1.4	20	1	5	5	78	7	11	21
28	11.6	33.7	1.6	22	4	8	12	174	20	9	16
29	16.2	21.1	1.3	16	5	7	13	89	9	10	18
30	13.7	26.7	1.8	15	9	9	18	113	11	10	21
31	16.2	17.8	1.0	18	0	4	4	116	11	11	14
32	18.5	34.5	2.1	16	4	6	9	99	10	10	29
33	13.1	31.3	1.5	21	6	9	15	98	10	10	16
34	12.3	20.2	1.2	17	2	5	8	95	9	11	14
35	11.3	17.7	1.2	15	1	4	5	97	9	11	17
36	15.6	14.9	1.0	16	1	2	4	61	6	10	12
37	25.9	77.4	4.0	19	6	6	12	230	21	11	39
38	12.8	36.6	2.1	18	3	3	6	148	13	12	21
39	14.8	22.9	1.2	20	2	10	12	96	10	10	11
40	18.5	32.4	1.8	18	5	6	10	112	10	11	20
41	12.0	10.1	0.6	16	1	5	6	85	11	7	13
42	6.7	35.2	1.8	20	8	10	18	149	19	8	23
Mean	12.1	37.7	3.0	14	8	7	15	307	25	12	33
SD	4.2	17.8	2.0	4	8	4	11	258	18	3	20

Table 2 Mean values (sd) for mineralization rates, biomass and C:N ratio of the biomass for different classes of grassland age. Significant differences between age classes are indicated by different characters within a column

Age class	n	Mineralization rates g Nm^{-2} wk^{-1}	Biomass g (d.m.) m^{-2}	C:N in biomass
1–2 years	9	0.92 (0.31)^{a}	527 (221)^{a}	26 (7)^{a}
3–5 years	9	1.15 (0.43)^{ab}	660 (234)^{a}	24 (6)^{a}
6–10 years	12	1.54 (0.42)^{b}	818 (303)^{b}	25 (6)^{a}
11–19 years	6	1.23 (0.44)^{ab}	959 (298)^{b}	28 (6)^{a}
>20 years	6	2.08 (0.66)^{c}	695 (82)^{ab}	25 (4)^{a}

and clay soils (1.5±0.6 g N m^{-2} wk^{-1}), and were not related to the soil textural classes (Table 1). Though very variable inside a class of age, the mineralization rates tended to increase from short term- (1–2 years) to mid term (3–5 years and 6–10 years) grasslands, but decreased in long term grasslands (10–19 years) and were highest after destruction of old grasslands (≥20 years) (Table 2). When all samples were used, a significant positive relationship between N mineralization and grassland age was observed. This relation was not significant when only grassland younger than 20 years were considered.

Biomass parameters

Grass biomass (roots and stubbles combined) after cutting varied widely among fields (Table 1). However, this might partly have been caused by differences in the height of the stubble (which was not recorded). On average, the amount of N in the biomass equalled 11 and 13 g N m^{-2} in clay soils and sandy soils, respectively. C:N ratios of the biomass (Table 1) varied widely (between 13 to 39). This might have been caused by different stubble to root ratios (not analysed, as stubbles and roots were processed jointly). There was no clear effect of grassland age or soil type on the C:N ratio of the remaining biomass (Table 2).

Soil parameters

Organic matter content of the soil varied from 10 to 77 g C kg^{-1} (Table 1) Although C contents of the old grasslands on clay soil were relatively high (>67 g kg^{-1}), there was in general no relation between C content and age of the grassland. In clay soils initial DOC was strongly related to total C, and DON to total N, while in sandy soils these relationships were less pronounced. In sandy soils the C:N ratios was generally higher (average of 18) than in clay soils (on average 11).

Contents of DON and DOC were lower in the sandy soil than in the clay soil (Table 1). During the 12 week incubation, DON and DOC content in the sandy soils did not change. In clay soils, DOC slightly decreased during the first 2 weeks while the grass died, but remained constant during the incubation period. DON decreased from 36 mg N kg^{-1} (±18 mg N kg^{-1}) in the non-treated samples to 26 mg N kg^{-1} (±14 mg kg^{-1}) after 12 weeks of incubation.

Indicators for net N-mineralisation

Soil and plant biomass parameters were tested as possible indicators for mineralization, both for the complete data set and for sandy soils and clay soils separately (Table 3 and Fig. 1). The regression lines for clay soils showed a better fit (i.e. higher R^2) than

Table 3 Single regression analyses with net mineralization as dependent variables (y in g N m^{-2} wk^{-1}) and the potential indicators as independent variables (x)

Potential indicator	Clay soil fitted line	R^2	Sandy soil fitted line	R^2	All samples fitted line	R^2
Biomass N (g N m^{-2})	n.s		y=0.056 x+0.450	0.38	y=0.043 x+0.840	0.10
Total C (g C kg^{-1})	y=0.027 x+0.260	0.59	y=0.016 x+0.730	0.22	y=0.022 x+0.522	0.48
Total N (g N kg^{-1})	y=0.258 x+0.423	0.66	y=0.339 x+0.620	0.28	y=0.204 x+0.749	0.50
C/N	y=−0.358 x+5.600	0.67	n.s		y=−0.063 x+2.250	0.17
DOC (mg kg^{-1})	y=0.002 x+0.676	0.52	y=0.004 x+0.698	0.16	y=0.001 x+0.940	0.38
DON (mg kg^{-1})	y=0.021 x+0.752	0.38	n.s		y=0.018 x+0.919	0.32
DOC/DON	y=0.101 x+0.190	0.23	n.s		y=0.101 x+0.200	0.23
hot-KCL (mg NH4-N kg^{-1})	y=0.023 x+0.507	0.59	y=0.033 x+0.547	0.24	y=0.020 x+0.712	0.47

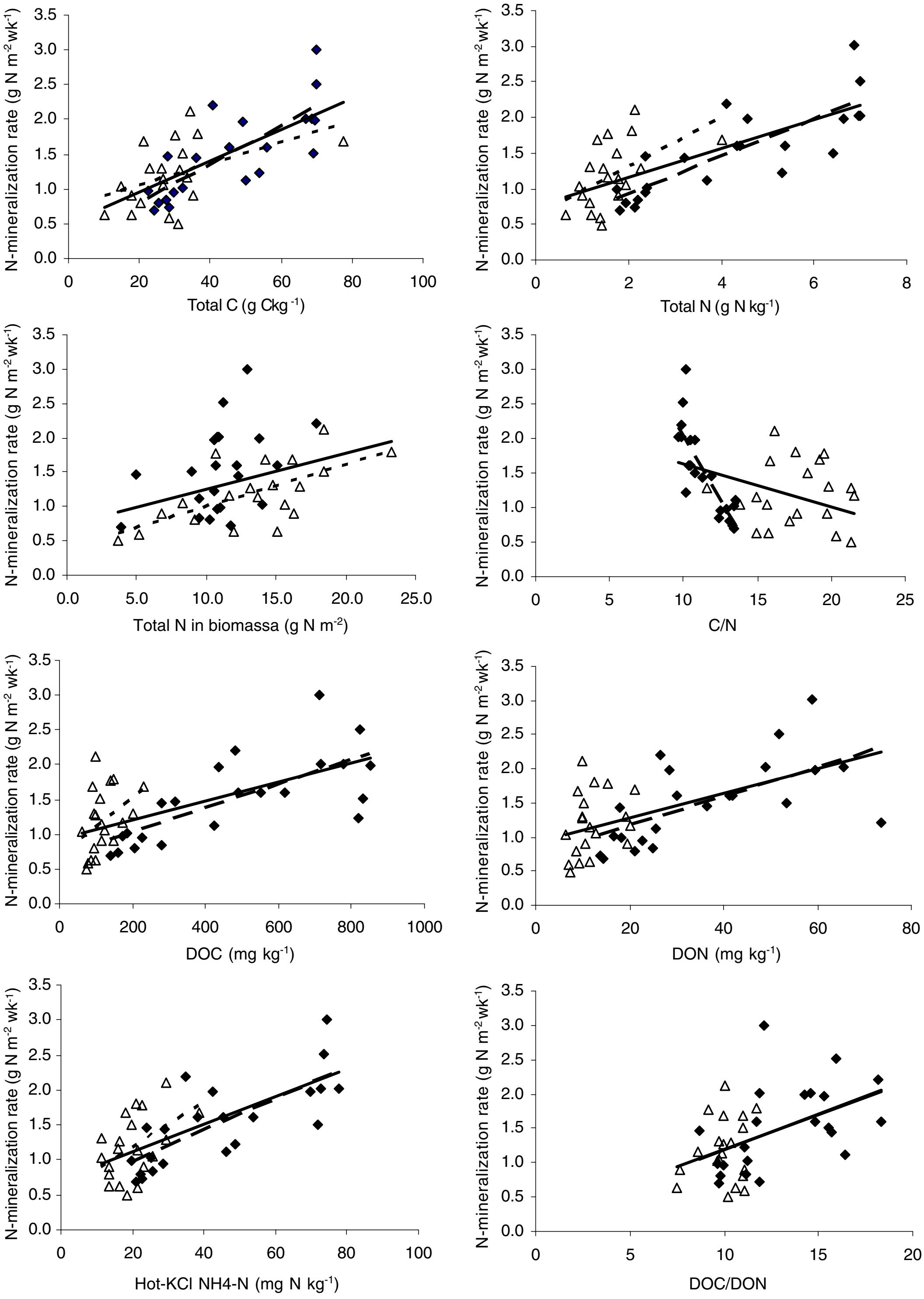

Fig. 1 Net mineralization rates (g m^{-2} wk^{-1}) in relation to potential indicators. Only significant relations (see Table 2) are presented as lines (Δ: sandy soils; ♦: clay soils; fitted lines: - - - - - sandy soils; — — —: clay soils: ▬▬▬ all samples)

for sandy soils. When data of both soil types were combined, all indicators in Table 3 were significantly related to net N-mineralization, although there was a large difference in fit between the indicators. Total C, total N, DOC and hot-KCl extractable NH_4^+ were considered good predictors, as these indicators were significantly related with N mineralization for both soil types (Table 3). Total N appeared to be the best indicator for both soil types and the combined data. Total C and hot-KCl extractable NH_4^+ performed second best. DON was significantly related with N mineralization for clay soils and the combination of both soil types, but not for sandy soils.

Since correlations among soil parameters were significant (data not presented), only one soil parameter at a time was included in the multiple regression analyses. So several stepwise multiple regressions were conducted, each with a combination of one soil parameter and either biomass N, age (log transformed), texture or soil type were tested (Table 4). Total soil N again gave best results. Including grassland age and plant biomass N improved the fit of the model, but hardly decreased the standard error of the estimate. With total N in soil, grassland age and N contents in the remaining biomass after cutting (roots and stubbles), 62% of the variance in N mineralization rate could be explained. Including soil type or texture class in the multiple regression analyses did not improve the results.

Discussion

Nitrogen mineralization

The first aim of this study was to quantify the potential mineralization after the grass was chemically killed. Nitrogen mineralization ranged from 59 to 361 kg N per ha in 12 weeks. Possible differences in moisture contents were not expected to cause differences in N mineralization since N mineralization is at maximum at a relatively broad range of moisture contents around field capacity (e.g. Antonopoulos 1999) and as long moisture content is at least 50% of field capacity, it is not a major factor controlling the mineralization rates (Gonçalves and Carlyle 1994). Only at dry or very wet conditions, a significant decrease in N mineralization may occur and those conditions were avoided. Mineralization rates were obtained at relatively high temperatures (20°C). The average temperature in the period half May to half August in the Netherlands is 16°C. In this period N uptake by the crop is highest. For a good comparison of the laboratory results with field conditions a correction factor of about 0.75 is appropriate, assuming a Q_{10} relationship of 2 (i.e. the mineralization increases with a factor 2 when temperature increases with 10°C; Stanford et al. 1973). Thus, the N mineralization corrected for field temperature ranges from 45 to 275 kg N ha^{-1} in the 12-week period of the incubation experiment.

Table 4 Results of multiple regression analyses with net mineralization in all samples (n=42) as dependent variables (y in g N m^{-2} wk^{-1}) and total N (g kg^{-1}), total C (g kg^{-1}), DON (mg kg^{-1}), DOC (mg kg^{-1}), and hot-KCl extractable NH_4^+ (mg N kg^{-1}) in combination with ln-transformed age (years) and the N content in the biomass (g m^{-2}) as independent variable

Model	R^2	Standard error of estimate
y=0.750+0.204*Ntotal	0.50	0.41
y=0.543+0.160*Ntotal+0.187*ln(age)	0.59	0.37
y=0.288+0.171*Ntotal+0.128*ln(age)+0.027*Nbiomass	0.62	0.36
y=0.521+0.022*Ctotal	0.48	0.41
y=0.329+0.018*Ctotal+0.204*ln(age)	0.59	0.37
y=0.209+0.018*Ctotal+0.18*ln(age)+0.013*Nbiomass	0.60	0.37
y=0.920+0.018*DON	0.32	0.47
y=0.612+0.013*DON+0.241*ln(age)	0.48	0.42
y=0.307+0.015*DON+0.170*ln(age)+0.031*Nbiomass	0.52	0.41
y=0.941+0.001*DOC	0.38	0.45
y=0.637+0.001*DOC+0.227*ln(age)	0.52	0.40
y=0.335+0.001*DOC+0.161*ln(age)+0.030*Nbiomass	0.56	0.39
y=0.714+0.020*hot-KCL NH4	0.47	0.42
y=0.505+0.015*hot-KCL NH4+0.197*ln(age)	0.57	0.38
y=0.213+0.017*hot-KCL NH4+0.130*ln(age)+0.030*Nbiomass	0.60	0.37

Since we found no other studies on mineralization rates in chemically killed but otherwise undisturbed swards, it is not possible to compare these figures to other studies that were comparable in setup. However, N mineralization rates in our study were in range of data from studies where grasslands were ploughed (Aarts et al. 2001; Johnston et al. 1994; Vertès et al. 2007; Whitehead et al. 1990). Those studies report a wide range of annual N mineralization rates (100 to 400 kg N ha^{-1} yr^{-1}) in the first year after grassland ploughing. This wide range may be due to large differences in experimental conditions (soil type, soil organic matter content, N management, sward age, crop type and management) and in the method of estimation of N mineralization (N balance, models, N uptake, and in-situ or laboratory incubations). High mineralization rates in our study and the linear increase in mineral N during the incubation period may indicate that we only determined the peak of N mineralization during several months after killing the sward. This is comparable to Vertès et al. (2007), who indicated that the N mineralization kinetics after ploughing of grassland consists of a first phase of several months with rapid and high N mineralization, followed by a period with much smaller N mineralization rates. This effect remained significant for several years, depending on the age the grassland.

It was assumed that only chemically killing the grass might reduce the risk of N losses, since ploughing is omitted. The effect of ploughing may be described as a combination killing the sward, chopping of the sward residues into smaller pieces, better mixing the sward residues in the soil and better aeration of the soil, but also disturbance of macro- and micro aggregates (Six et al. 2004). However, the high N mineralization rates in our experiment suggests that killing of the sward is a major factor controlling N mineralization in destroyed grasslands. This last assumption is supported by results of Velthof et al. (2009), who showed that mineral N contents in the soil were similar or higher after only chemically killing of the swards than after chemically killing and ploughing of the sward. Arnott and Clement (1966) found that N yields of crops following grassland that was chemically killed (and not ploughed) were similar to those obtained by ploughing (and not chemically killed). Both studies suggests that soil cultivation after chemically killing of the sward does not further enhance N mineralization.

Relation between indicators and N mineralization

The results show that the contents of total N, total C, DOC and hot KCl NH_4^+ were the best indicators of the N mineralization. Accoe et al. (2004) in Belgium and Hassink (1995) in the Netherlands showed that total N is also a good predictor of N mineralization of undisturbed, but intensively managed grassland. It is therefore used in N fertilizer recommendations for grassland in the Netherlands. In a simple regression model for predicting the N off take in newly resown grassland (Hatch et al. 2004), total N content was also a significant parameter, the other parameters being hot KCl NH_4^+, the clay content and the accumulated daily temperature. Like Accoe et al. (2004) we found a negative relationship between the C:N ratio and the N mineralization, for the clay soil and the combined data. In this study we only tested rapid chemical methods as indices for mineralization. Our results and conclusions only apply to these methods and not to other measurements (e.g. on soil microbial biomass).

The results of the multiple regression analysis showed that when the N content of the roots and stubbles and the number of years since sowing is known, the prediction of N mineralization could be improved up to 62%. (Table 3). Although Johnston et al. (1994) and Vertès et al. (2007) also reported a tendency that N mineralization increases with increasing age of ploughed grassland, we have to note that this only applied when all samples were included. In field situations it is unlikely that those very old grassland will be destroyed for renovation. Although we found that the prediction of N mineralization, could be improved when the N content of the biomass (both roots and stubbles) is known, it is a too difficult and time-consuming method to suggest its determination as a standard and rapid method in laboratories to provide farmers fertilizer recommendations on basis of soil and plant tests.

Performance of DON as indicator for N mineralization

Based on many studies (Appel and Mengel 1990, 1998; Bregliani et al. 2006; Groot and Houba 1995) and an experiment in permanent (not killed) grasslands on sandy soils in the Netherlands (Velthof 2003), we expected DON (i.e. 0.01 *M* $CaCl_2$ extractable organic N) to be a good index for mineralizable N. However, the exact role of DON in mineralization is not clear. Mengel et al. (1999) indicated that determination of the N compounds in $CaCl_2$-extractable organic N may improve its use as an index, because amino N and the amino sugar N were positively correlated with N mineralization. In our experiment DON was significantly related with N mineralization in the clay soil and the overall dataset, but not for the sandy soil. We also expected that DON and DOC contents would temporary increase, because of decaying grass residues like in studies of Bhogal et al. (2000) and Murphy et al. (2007), who showed flushes of DON after residue incorporation. In our experiment, the DON contents in sandy soils remained constant during the incubation and decreased slightly in the clay soils.

Obviously, the role of DON in mineralization and processes controlling DON contents in the soil are yet unclear. Possibly, DON is a better predictor for N mineralization in sieved soil samples as in the study of Velthof et al. (2009), than in undisturbed samples containing crop residues. Because of the promising results of DON as indicator for mineralization in many studies and the fact that measurement of DON is a rapid and easy method, further study on this topic is needed.

Variability in mineralization and indices

Although total N, total C, DOC and hot KCl NH_4^+ were significantly related to the N mineralization, the R^2 of 40–60% indicated that a considerable part of the variance in N mineralization is not explained. Even when some additional indicators like age or biomass N were included, the explained variance never exceeded 62%. There are several possible causes for this. First, N mineralization is a complex process in which many biological, chemical, physical factors play a role. A practical (and therefore necessarily simple) mineralization index can never account for all these factors and thus part of the variance in N mineralization will always be unaccounted for. Secondly, spatial variability of N parameters may have played a role. Since we measured the N indicators in one column, they may not always apply to mineralization rates derived from measurements in the other column. A third complicating factor may be the variability within the columns. We used undisturbed sward samples where the killing of the grass sward may have introduced a significant source of variability by mineralization of crop residues. Since determination of N mineralization was based on small soil cores samples, the results may deviate from studies in which incubations are carried out with well mixed composite soil samples.

Some studies report higher correlations (up to 85%) between mineralization indices and measured soil mineralization (e.g Gianello and Bremner 1986; Groot and Houba 1995; Mengel et al. 1999), but in those studies the effects of small scale spatial variability are minimized by sieving and mixing the soil, which is by definition impossible in undisturbed soil cores. In some studies, the N yield from unfertilized grassland is used as an indicator of mineralization. The percentage of the variance in the N off take explained by mineralization indices is somewhat higher (60–90%) compared to our study (Hassink 1995: total N; Hatch et al. 2004: hot KCl NH_4^+). This is mainly because the N off take is measured by mowing of a relatively large grassland area, which reduces spatial variability of N mineralization compared to the small samples in our study. However, it must be noted that grass growth may be limited by climatic factors and that the N yield of grassland is only an indicator for part of the N mineralization. Large amounts of N (more than 100 kg N ha^{-1}; Whitehead et al. 1990) needed for the establishment of roots and stubbles are not taken in account.

Conclusion: To a field indicator for mineralization?

In our study we focussed on rapid chemical indicators for N mineralization in a laboratory experiment. Tested indicators can be used to predict N mineralization in order to better fine-tune N applications in crops after chemically killing the grass. The laboratory study showed that contents of total N, total C, DOC and hot KCl NH_4^+, whether or not in combination with the N content of the biomass and the number of years since sowing, are promising indicators for mineralization in these grasslands. Studies are needed to test the predictive value of these indicators in the field.. In our laboratory study, DON was not well related to N mineralization in chemically killed grassland. This is, in contradiction with other studies which showed that DON is a promising indicator for mineralization.

The high N mineralization rates (0.5 to 3.0 g N m^{-2} $week^{-1}$) in our experiment and results of the field study of Velthof et al. (2009), suggest that killing the sward is the major factor controlling N mineralization in destroyed grasslands. Soil cultivation after killing of the sward may not further enhance N mineralization. Further experiments, either in the laboratory or in the field, have to be carried out to get more insight in N mineralization in destroyed grassland.

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