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Incubation Experiments on Net Nitrogen Mineralization in Organic Greek Soils

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Abstract: Aerobic incubation experiments were conducted in organic surface samples collected from arable soils of Philippoi, in Northern Greece. Laboratory experiments were carried out to investigate the nitrogen (N) mineralized from soils and sugar beet residues incorporated into the soils. Cumulative mineralization of N, potentially mineralizable nitrogen (N_0), and mineralization rate constant k were estimated after 30 continuous incubation weeks at 35°C. Total N content of soils ranged between 6.6 and 19.2 g kg^{-1}, total soil carbon from 119 to 309 g kg^{-1}, soil organic carbon ranged from 119 to 308 g kg^{-1}, and the C : N ratio varied from 13.5 to 18.3. The cumulative net mineralized N ranged between 132 and 426 mg kg^{-1} for nonamended soil and between 165 and 586 mg kg^{-1} for residue-amended soil. Nitrate was the main form of mineralized N, although appreciable amounts of ammonium were measured. Potentially mineralizable nitrogen (N_0) varied between 254 and 1067 mg kg^{-1} for nonamended soil and 311–1465 mg N kg^{-1} in residue-treated soil. The mineralization constant k was between 0.052 and 0.068 ($week^{-1}$). Close relationships between total soil N and soil organic carbon, and between cumulative mineralization and total soil N were found. Mineralization occurred rather rapidly, although the amount of mineralized N per week was reduced in the later weeks of incubation. Variation in net mineralization among soil samples can be attributed to soil organic matter content, origin, and state of decomposition, as well as differences in management histories,

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and soil and microenvironmental conditions. Monitoring of mineralization process may ensure a basis for increasing nitrogen use efficiency.

Keywords: Nitrogen, mineralization, ammonium, nitrates, organic soils

INTRODUCTION

Methods to determine the potential N mineralization include laboratory incubations, chemical extractions, measurements of N mineralization in the field, and N^{15}-labelled fertilizer techniques (1). It is widely recognized that incubation tests are suitable for comparative estimations in different soils (2). Field and laboratory studies have revealed a multitude of soil factors that affect mineralization processes, such as quality of organic amendments, C : N ratio, temperature, disturbance of soil horizons, and soil moisture content (3–6). Of these factors, N mineralization appears to depend mainly on temperature, moisture, aeration, acidity, form, and content of soil organic matter (7–9). Soil inorganic N may originate from fertilizers, plant residues remaining in the fields after harvest, atmospheric deposition, and animal sources. Nitrogen availability also changes with time and depends on residual N content, mineralization potential, and the amount immobilized during decomposition of organic residues. Mineralization of N at time t can be determined by establishing incubation experiments of soils under controlled conditions in the laboratory.

The main objective of this study was to measure N mineralization in organic soils located in the area of the Philippoi peatland, Northern Greece (Fig. 1), and to determine the changes of net mineralization and kinetic parameters after incorporation of dried sugar beet residues in incubated soils. Sugar beets are widely cultivated in the studied area, and considerable quantities of residues after harvesting can be incorporated into the soils. The amount of sugar beet residues range from 26.2 to 36.3 tones per hectare (unpublished results) Application of N fertilizer may be reduced because these residues are a source of inorganic nitrogen by means of the N mineralization process.

MATERIALS AND METHODS

Samples from organic soils, classified as *Histosols* (10), were collected from a depth 0–30 cm in the area of Philippoi, Northen Greece (Fig. 1), which covers about 9000 h. The district is extended from East to West 24° 20′ 23″ and 24° 04′ 41″ and from North to South 41° 02′ 05″ and 40° 55′ 33″, respectively. After air-drying and sieving, chemical analysis were performed on fine earth (soil fractions <2 mm). Sugar beet leaves were collected and washed

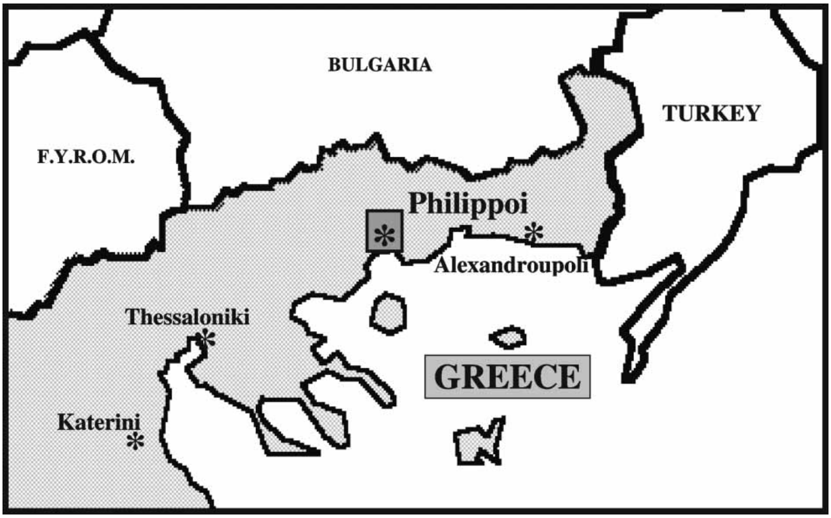

Figure 1. Simplified map of northern Greece.

with distilled water, dried at 60°C for 48 h, and then pulverized. Soil pH values were measured in a 1 : 1 soil to H_2O suspension (11). Soil carbonates were determined by the volumetric calcimeter method (12). Total soil carbon and total nitrogen were determined by LECO Elemental Analyzer (Model CNS-2000). Organic carbon content was estimated as the difference between total and inorganic form. Homogeneous subsamples were obtained from each air-dried soil sample, and nitrogen mineralization was estimated under controlled conditions by the incubation method of Stanford and Smith (13). The incubation procedure involved the addition of triplicate 15 g air-dried soil samples mixed with equal quantity of quartz sand (20 mesh). Treatments for all soil samples were (1) nonamended soil and (2) soil amended with dried sugar beet residue. For soils receiving residue, dried and pulverized sugar beet leaves (0.2 g) were added to each incubation tube and mixed thoroughly. Soil tubes were placed to the incubator in vertical position in the dark. Soil water content was monitored by weighing the samples three times a week and adding water up to field water capacity (excess water was removed under vacuum 60 cm Hg) (13). The temperature during the period of incubation was kept at 35°C, and N mineralization was determined at time intervals of 2, 4, 7, 10, 14, 18, 24, and 30 weeks. Leaching was performed by adding 100 mL of 0.01 M $CaCl_2$ followed by 25 mL of N-free nutrient solution. The leachates were collected in glass beakers of 100 mL, and the total volume was measured. After each incubation, NO_3-N and NH_4-N were determined by a FIAstar 9000 Analyzer (FOSS TECATOR, Sweden). The nitrogen mineralization potential (N_0) was estimated from the equation $N_t = N_0$

$(1 - e^{-kt})$, where N_t is the cumulative amount of N mineralized during a specific time interval (t) in weeks, N_0 is the N mineralization potential, and k is the rate constant (13). N mineralization between the two treatments of each soil sample were compared with the Student's pairwise *t*-test, and differences were deemed statistically significant at $p < 0.05$ and $p < 0.01$ probability level.

RESULTS AND DISCUSSION

Some characteristics of the examined soils are presented in Table 1. Soils were slightly acid or slightly alkaline and rich in total soil carbon (119–309 g kg^{-1}). Organic carbon ranged from 119 to 308 g kg^{-1}, total soil N was between 6.6 and 19.2 g kg^{-1}, and the C : N ratio varied from 13.5 to 18.3. These values are rather high in comparison with those obtained by typical arable Greek soils in which the C : N ratio ranged from 5.3 to 11.3 (14). A strong relationship was found between total soil nitrogen and soil organic carbon ($R^2 = 96.0$, $n = 10$).

The cumulative amounts of mineralized N from the soils and soil/residue mixtures are presented in Table 2. The N_0 ranged from 254 to 1067 mg kg^{-1} in nonamended soils, whereas the N_0 values were greater in residue-amended soils, ranging from 311 to 1465 mg N kg^{-1}. Statistical analysis showed that addition of residues significantly increased mineralized N pools (cumulative and N_0) in all samples (Table 2). Mineralized N derived from sugar beets varied widely and ranged from 13 to 160 mg N kg^{-1}, with a mean value 63 mg N kg^{-1}. Overall, mineralized N was a small percentage (1.20–2.34%) of the total soil N in the nonamended soils, and these values

Table 1. Soil characteristics and parameters of the examined soils

Samples	pH	Total carbon (g kg^{-1})	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	C : N
P3	6.9	119	119	6.70	17.8
P4	7.4	220	214	11.7	18.3
P7	7.4	256	245	14.6	16.8
P8	7.4	268	265	16.7	15.9
P12	7.5	210	200	12.1	16.5
P13	7.6	175	118	6.60	17.8
P14	7.6	181	131	8.50	15.4
P15	7.7	171	153	11.3	13.5
P16	7.2	309	308	19.2	16.0
P17	7.3	294	286	18.2	15.7

Table 2. Kinetic parameters of net nitrogen mineralization in nonamended soils (S) and soils amended with sugar beet leaves (S + R). Soils were incubated at 35°C for 30 weeks

	Cumulative N mineralization ($mg\,kg^{-1}$)			NO_3-N ($mg\,kg^{-1}$)		NH_4-N ($mg\,kg^{-1}$)		Constant k		No ($mg\,kg^{-1}$)			$N_{cum}/N_{tot} \times 100$ (%)	
Samples	(S)	(S + R)	SSD[a]	(S)	(S + R)	(S)	(S + R)	(S)	(S + R)	(S)	(S + R)	SSD	(S)	(S + R)
P3	132	175	**	101	159	31	16	0.054	0.055	254	340	**	1.97	2.61
P4	211	234	**	176	210	35	24	0.057	0.057	418	463	*	1.80	2.00
P7	201	267	**	172	237	29	30	0.055	0.056	375	496	**	1.38	1.83
P8	200	273	**	174	244	26	29	0.059	0.060	407	527	**	1.20	1.63
P12	192	248	**	162	222	30	26	0.056	0.057	362	459	**	1.59	2.05
P13	152	165	**	133	141	19	24	0.052	0.054	264	311	*	2.30	2.50
P14	178	217	**	147	200	31	17	0.058	0.060	350	413	*	2.09	2.55
P15	216	242	*	180	223	36	19	0.057	0.058	431	471	*	1.91	2.14
P16	395	526	**	314	495	81	31	0.066	0.066	928	1,210	**	2.06	2.74
P17	426	586	**	323	555	103	31	0.068	0.068	1,067	1,465	**	2.34	3.22

[a]SSD, statistically significant differences exist between treatments at $^{*}p < 0.05$ or $^{**}p < 0.01$, according to pairwise Student's *t*-test.

increased to 1.63–3.22% after incorporation of plant residues (Table 2). Our findings agree with results of other researchers (15) who reported that 1–4% of total N was mineralized per year, and that total N can be used to predict roughly the N mineralization potential of soil. These differences may be related to N soil content and moreover to the organic matter content (16, 17).

The highest N mineralization values (526 and 586 $mg\,kg^{-1}$) were recorded in samples P16 and P17 (Table 2). It should be noted that part of the surface layers in these soils were affected from burning some years ago. Increased N mineralization values in response to burning have been reported elsewhere (18). High mineralization values in these samples may also be due to specific cropping systems or quality of the soil organic matter, as was the case in another study (19). The C : N ratio of soil is also an important factor, which affects mineralization. Immobilization of soil N is favored when the ratio is >20, whereas mineralization occurs when C : N is <20 (20). Contrary to reported results, (21), C : N and total N were not related in the present study and may be attributed to low range of C : N values.

Total nitrogen content in sugar beets was found to be 34.9 $g\,kg^{-1}$, and plant residues with N concentration $>17.0\,g\,kg^{-1}$ are considered to be desirable for N mineralization (22). The main form of mineralized nitrogen was NO_3-N, although appreciable amounts of NH_4-N form were measured (Table 2). Presumably, NH_4-N is oxidized and is converted rapidly into NO_3-N. The observed N mineralization potentials in the residue-amended soils were significantly higher than in nonamended soils (Table 2). Neglecting the magnitude of cumulative net mineralized N, residues showed a similar pattern of N released with time (first-order kinetics), and in this study, additional mineral N accumulated in soils. Immobilization was not observed in the residue-amended soil samples, indicating that soil microorganisms can easily decompose the incorporated material. It is well documented (23) that the conversion of inorganic N to organic N compounds and the reverse process of mineralization occurs simultaneously. Other researchers (24) concluded that immobilization occurs mainly at the initial stages of incubation experiments carried out with sugar beet residues. However, in their study, the plant material was not air-dried and ground, and the total nitrogen content was much lower.

The mean constant k was 0.058 per week and ranged between 0.052 and 0.068 per week, whereas a slightly greater average value of 0.059 was found in the residue-treated soils (0.054–0.068 range) (Table 2). Typically, k values will vary due to changes in organic matter content and degree of decomposition, or to factors that favor or inhibit microbial activity. Other authors have reported higher k values in incubation experiments conducted in inorganic soils that had also been amended with equal quantity of sugarbeet residues (25).

Strong relationships were also found between the cumulative mineralized soil nitrogen and N_0 in the case of soil incubation, as well as between N_0 in the amended soils and N mineralization. These are described by the following equations:

$$Y_{Nmin.} = 58.4885 + 0.3538X_{N0} \quad (R^2 = 99.3^{***}, n = 10)$$
$$Y_{N0(sugarbeet)} = -179.562 + 2.7107X_{Nmin.(sugarbeet)} \quad (R^2 = 99.1^{***}, n = 10)$$

Similar relationships have been reported by other authors (26, 27). The results of this study also indicated that cumulative N mineralization was influenced by both soil organic carbon and total soil nitrogen. These relations are described by the following equations:

$$Y_{Nmin.} = 8.0061 + 1.0902X_{Org.Carbon} \quad (R^2 = 61.8^{**}, n = 10)$$
$$Y_{Nmin.} = 6.2511 + 17.8383X_{Ntot.} \quad (R^2 = 67.5^{**}, n = 10)$$

Nitrogen mineralization can strongly be affected by soil disturbance, as was the case in the present study (4, 15). It should be stressed that this study was carried out under controlled conditions (i.e., constant temperature and moisture), and plant materials were ground and well mixed with soil. Environmental conditions in the field will be more variable, and thus decomposition rates will vary as microbial populations respond to their environment. Hence, N mineralization in the field may not be accurately estimated by incubation studies.

The potentially mineralized nitrogen between both treatments is expreseed by the following equation:

$$Y_{N0(residue\ amended)} = -63.5049 + 1.3983_{N0(nonamended)}$$
$$(R^2 = 99.0^{***}, n = 10)$$

It is assumed that N_0 of a soil can be a definable quantity useful for estimating the N-supplying capacities of soils under specific environmental conditions (28). This is very important especially in arid and semiarid arable areas where soils are poor in N and soil organic matter. The incorporation of plant residues into soils enhances the ability of soils to supply inorganic nitrogen to crops. Nowadays, this property is being exploited in practice by incorporating plant residues into soils after harvesting. It is well known that plant nutrition demands can partially be satisfied in the next period by means of this process. Apart from the improvement of soil properties, farmers could profit by incorporation of plant materials through decreased cost for buying inorganic N fertilizers.

CONCLUSIONS

Properties of the studied soil samples greatly differ. Net N mineralization (NO_3-N and NH_4-N) also varied among soils during the incubation period. Soil organic matter quality and content, cropping history, and climatic factors were presumably responsible for decomposition rates. Nitrate was the main form of mineralized N. It is concluded that under prevalent conditions, ammonium can be oxidized and rapidly converted into nitrate. The quantity of mineralized N per week decreased with time. Relatively large amounts of mineralized N were recorded in samples collected from sites affected by burning; thus, easily decomposable materials may cause an increase in N mineralization. N mineralization of sugar beet residues demonstrated a first-order kinetic pattern over time. Net immobilization was not observed in samples amended with residues, indicating that the incorporated material can easily be mineralized by soil microorganisms. Relationships were found between total soil nitrogen and soil organic carbon, between the mineralized nitrogen and N_0, and between total soil nitrogen and N_0. The potentially mineralized soil N can be a useful parameter for estimating the N-supplying capacities of soils. This is crucial in arid and semiarid arable areas where soils are poor in N and soil organic matter. In practice, mineralization should be taken into account by means of residue incorporation into soils, after harvesting for partial satisfaction of plant demands. In addition, soil properties can be improved, and the cost for buying N fertilizers may substantially be reduced.

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