Organic and inorganic nitrogen leaching from incubated soils subjected to freeze-thaw and flooding conditions

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Wang, F. L. and Bettany, J. R. 1994. **Organic and inorganic nitrogen leaching from incubated soils subjected to freeze-thaw and flooding conditions.** Can. J. Soil Sci. **74**: 201–206. Freeze-thaw and flooding of usually well-drained soils occur in the spring in the prairie and boreal regions of Canada. We studied the impact of these conditions on nitrogen leaching in a Black Chernozemic soil (Udic Boroll). Soil samples, subjected to different treatments, were incubated for 12 wk in the laboratory and leached every 2 wk with 0.001 M $CaCl_2$ solution. The cumulative leaching loss of total N (mg kg^{-1} soil) was reduced by freeze-thaw (76.0), flooding (41.4) and a superimposition of the two treatments (28.8) compared to the control (109). All treatments affected the distribution of the forms of N leached. The total loss of **water soluble organic N (SON)** and ammonium-N was in the order of flooded > flooded-freeze-thaw > freeze-thaw = control. In the leachates from the flooded treatments, SON accounted for 71.5–77.4% of the total N leached. Nitrate- and nitrite-N dominated the total leachable N in the unflooded treatments following an order of control > freeze-thaw > flooded = flooded-freeze-thaw. During the incubation, the Eh of the flooded soils decreased from 344 to −46 mV, compared to a variation in Eh from 355 to 301 mV for the unflooded soils. The maximum rate of leaching of organic nitrogen from the flooded treatment (0.53 mg N kg^{-1} d^{-1}) coincided with a sharp decrease in Eh, from 131 to 42 mV. It is concluded that climatic events will have a significant impact on the dynamics of soil nitrogen. Flooding, in particular, may promote the loss of N in water soluble organic matter.

Key words: Flooding, freeze-thaw, organic and inorganic nitrogen leaching, redox potential

Wang, F. L. et Bettany, J. R. 1994. **Lessivage de l'azote organique et de l'azote minéral dans des sols incubés, soumis à des alternances de gel-dégel et à l'inondation.** Can. J. Soil Sci. **74**: 201–206. Les alternances de gel et de dégel et la submersion peuvent se produire au printemps, même dans les sols habituellement bien drainés des Prairies et des régions boréales du Canada. Nous avons étudié les incidences de ces conditions sur le lessivage de N dans un sol chernozémique noir (Boroll udique). Des échantillons de sol exposés à divers traitements étaient mis en incubation en laboratoire pendant 12 semaines et lessivés toutes les deux semaines avec une solution de $CaCl_2$ 0,001 M. Les pertes cumulatives par lessivage du N total, en mg kg^{-1} de sol, étaient diminuées par les alternances gel-dégel (76,0), par l'inondation (41,4) et par la combinaison des deux traitements (28,8) en regard du traitement témoin (109). Tous les traitements influaient sur la répartition des formes de N lessivées. Les déperditions de N organique soluble dans l'eau (Nos) et de N ammoniacal s'établissaient dans l'ordre suivant: inondation > alternance gel-dégel > gel-dégel = témoin. Dans le percolat des traitements sous inondation Nos représentait de 71,5 à 77,4% du N total lessivé. Les formes nitrate et nitrite étaient les fractions dominantes du N lessivé total dans l'ordre suivant: témoin > gel-dégel > submersion = submersion- gel-dégel. Au cours de l'incubation, les valeurs Eh des sols submergés tombaient de 344 à 46 mV alors que dans les sols non submergés, la chute n'allait que de 355 à 301 mV. Le taux maximum de lessivage du N organique dans les traitements avec inondation (0,53 mg N kg^{-1} j^{-1}) coïncidait avec une brusque chute de Eh qui passait de 131 à 42 mV. Il ressort de ces observations que les conditions climatiques exercent une importance significative sur la dynamique de l'azote dans le sol. L'inondation peut notamment entraîner des déperditions de N dans la matière organique hydrosoluble.

Mots clés: Inondation, gel-dégel, lessivage, N organique, N minéral, potentiel redox

The leaching loss of nitrogen from soil has been an agricultural and environmental concern. Evolution of gaseous forms of nitrogen (e.g. N_2O and N_2), leaching of nitrate and nitrite, losses of nitrogen through erosion and grain export are considered major pathways for soil nitrogen output (Frissel and van Veen 1978). Few studies have addressed the loss of soil nitrogen through other pathways. Recent studies at sites of deciduous forest in North Carolina and at the prairie-boreal forests transition in northern Saskatchewan suggested that leaching of nitrogen in water soluble organic form (SON) could be another important pathway for soil nitrogen loss (Ellert 1990; Qualls et al. 1991). Whereas the effect of soil management on the loss of SON has been shown in some Luvisols (Ellert 1990), the impact of climatic events such as freeze-thaw and flooding remains unknown. These processes may be of particular importance in the soil nitrogen cycle of the boreal region where most leaching to the water table is believed to occur in the spring following many freeze-thaw cycles.

Under aerobic conditions, nitrate nitrogen (NO_3-N) is the major product of N mineralization in soils whereas under anaerobic conditions, such as in paddy soils, accumulation of ammonium nitrogen (NH_4-N) predominates (Ponnamperuma 1972). Numerous studies have been conducted on the leaching of NO_3-N from soil (McNeal and Pratt 1978; Legg and Meisinger 1982). Little information, however, is available on the leaching of NH_4-N from soils, especially soils subjected to freeze-thaw and flooding conditions.

Table 1. Selected chemical and physical properties of the Black Chernozemic soil[z]

pH	5.8	Field capacity moisture content	341 g kg^{-1}
Total C	66 g kg^{-1}	Electrical conductivity	0.51 dS m^{-1}
Organic C	65 g kg^{-1}	NH_4-N	3.6 mg kg^{-1}
Total N	5.7 g kg^{-1}	NO_3-N	49 mg kg^{-1}

[z] The methods used: pH (soil:water = 1:1); electrical conductivity (aqueous extract of a saturated soil); field capacity moisture content (Klute 1986); total C and inorganic C (Tiessen et al. 1981, 1983); organic C (difference between the total C and inorganic C); total N (Thomas et al. 1967; Technicon Industrial Systems 1978); NH_4-N and NO_3-N (extracted from the soil with 2 M KCl in a ratio of 1:5 (soil:extractant), Technicon Industrial Systems 1973, 1978).

Table 2. Details of soil treatment imposed in each 2-wk incubation[z]

I. Control	Soil incubated at field capacity moisture content at 23°C for 14 d
II. Freeze-thaw	Soil incubated at field capacity moisture content at 4°C for 1 d, −10°C for 1 d, 4°C for 1 d, and 23°C for 11 d
III. Flooded	Soil incubated under saturation and flooded with 0.001 M $CaCl_2$ solution at 23°C for 14 d
IV. Flooded-freeze-thaw	Soil flooded as in treatment III incubated as described in treatment II

[z] Ottawa sand (0.53–0.80 mm, Fisher Scientific No S-23) washed with 0.1 M HCl and deionized water was used in the study as blanks of the above four treatments.

The objectives of this study were, therefore, to examine the effects of freeze-thaw and flooding conditions on the leaching of organic and inorganic nitrogen from a usually well-drained soil using a leaching-incubation technique in the laboratory.

MATERIALS AND METHODS

A silty clay Black Chernozemic soil (Udic Boroll), taken from a well-drained site at Melfort, Saskatchewan, was used in the study because of its relatively high organic matter content and dominance in the region. The soil had been under a wheat-fallow rotation for about 4 yr. The A horizon (13 cm) of a fallow field was sampled, air-dried, put through a 2-mm sieve, and used in subsequent study. Selected chemical and physical properties of the soil are presented in Table 1. Soil treatments applied in the incubation-leaching study are listed in Table 2. The soil was leached at intervals of 2 wk during an incubation time of 12 wk.

The incubation-leaching techniques of Stanford and Smith (1972), as modified by Wang and Bettany (1993), were used in the study. The soil sample (50 g, oven-dry basis) was placed on a glass fibre filter paper (1.6-μm effective retention, Whatman GF/A) in an 8 × 5-cm polypropylene Buchner funnel with a detachable base. The bulk density of the packed soil in the funnel was 0.87 ± 0.07 g cm^{-3} before leaching. A thin glass wool pad was put on the top of the soil to prevent it from dispersing when solution was poured on the soil. Prior to incubation, a pre-leaching was carried out to minimize the influence of air-drying of the soil samples and of soluble nitrogen originally present in the soils on subsequent treatment effects. The air-dried soil sample in the funnel was initially leached by 120 mL of 0.01 M $CaCl_2$ solution and followed by 100 mL of 0.001 M $CaCl_2$ solution. Vacuum was used to remove excess solution until the field capacity of the soil was reached. For the control and freeze-thaw treatments, the funnel was placed directly into a 13 × 11-cm jar. For the flooded and flooded-freeze-thaw treatments, the funnel was first placed into a small cup which took little air-filled space of the jars, but enabled the soil to be saturated and flooded. The cup was then placed into the jar. Twenty millilitres of leaching solution was added to the cup and the funnel was placed in the cup. Sixty millilitres of leaching solution was then added to the funnel. The solution stood about 7 mm over the soil surface at the end of a 2-wk incubation.

The jars were sealed after leaching of the soil to minimize the loss of soil water. The incubation was conducted under room light (fluorescent) and at various temperatures according to the soil treatments. All the treatments were duplicated. During the incubation period, the loss of water from the soil was less than 0.1%.

Calcium chloride (0.001M) was used as a leaching solution, which kept the soil flocculated and minimized acid additions (Ellert and Bettany 1988). After each 2-wk period, the unflooded treatments (I and II) were leached with 100 mL of the solution in 10-mL increments over a period of 200 min. For the flooded treatments (III and IV), the saturating solutions in the soils were first collected as they flushed naturally during the leaching procedure. The flooding solutions remaining in the cups were then added to leach the soils. Finally, 20 mL of leaching solution was used. The leaching time for soils with the flooding treatments was the same as that for other soils (200 min). Vacuum was applied at the late leaching period to facilitate leaching, to collect excess leachates, and to bring the soils to field capacity moisture content.

The leachates were filtered through 0.45-μm membrane filters (HA-Millipore). Total N, including nitrate and nitrite nitrogen, was determined using a potassium persulfate digest and reduction by Devarda's alloy (Reveh and Avnimelech 1979). Ammonium nitrogen in the leachates was measured by Technicon AutoAnalyzer using industrial method 329-74W/B (Technicon 1978). Hydrolysis of amino acids is virtually eliminated in this nitroprusside catalyzed reaction in a working buffer solution of low alkalinity (16 g NaOH L^{-1}) at a low reaction temperature (37°C). Nitrate and nitrite nitrogen were determined together (NO_3-N+NO_2-N) by continuous flow analysis (Technician Industrial Systems 1973). Water soluble organic nitrogen was calculated as the difference between the total and the inorganic N.

The redox potentials (Eh) of the control and flooded treatments were measured at room temperature (23 ± 0.5°C) using a combined platinum and AgCl (reference) electrode attached to a pH meter (Fisher Model 825 MP). The Eh measurement was carried out every 2–3 d as well as at the time immediately before and after leaching of the soils. The electrode was calibrated using a standard Eh solution which contained 0.1 M $Fe(NH_4)_2(SO_4)_2$, 0.1M $FeNH_4(SO_4)_2$ and 1 M H_2SO_4 (Light 1972). In the measurement, the electrode was inserted at random into the soil at a depth of about 2.5 cm.

Table 3. Cumulative losses of various N components from the soil treatments in six 2-wk incubation-leaching sequences

Soil treatment	Organic N	NO_3-N+ NO_2N	NH_4-N	Total leachable N^z
		(mg kg^{-1})		
Control	11.4	97.9	0.04	109
Freeze-thaw	13.7	62.2	0.03	76.0
Flooded	29.6	0.9	10.9	41.4
Flooded-freeze-thaw	22.3	1.5	5.0	28.8
LSD (0.01)	5.5	2.4	2.1	6.3

^{z}Total leachable N = sum of organic N, NO_3-N+NO_2-N and NH_4-N.

The emf was recorded for at least 10 min. so that a relatively steady (Δ emf/$\Delta t \leq 2$ mV min^{-1}) reading was obtained. Two soil replicates were used in the Eh measurements. The errors of the measurement were less than ±9 and ±4 mV for the control and flooded treatments, respectively. During 12 wk of incubation, the pH of the flooded soil changed from 5.8 to 6.9 whereas the Eh changed from 344 to −46 mV. Thus, the measured Eh mainly reflected the change in redox potentials of the soil since the observed pH change would only cause approximately a 50 mV drop in Eh (Lindsay 1979). The obtained Eh was a mixed potential in the systems studied — a weighted average of the potentials of all the redox couples present (Bohn 1968).

All results are presented as means of duplicated treatments. Statistical analyses on the difference between the means of the treatments followed the LSD procedure of Steel and Torrie (1980). Standard errors of means were used to indicate the errors of average rate data obtained in each two-week incubation period.

RESULTS AND DISCUSSION

Leaching of Organic Nitrogen

Compared to the control, flooding significantly increased the total leaching loss of SON from the soil whereas freeze-thaw alone did not (Table 3). Moreover, SON leached from the flooded-freeze-thaw treatment was significantly lower than that from the flooded treatment. These results must be considered as net effects of the processes that promote and depress the release SON over the cumulative time period.

Processes primarily contributing to the production of soluble organic matter include: substrate fragmentation, depolymerization and solubilization (Reid et al. 1982; Beckwith and Butler 1983), microbial lysis in inhospitable environments (Lynch 1982), and faunal grazing on soil microbes (Huhta et al. 1988). Freeze-thaw conditions will lyse microbial cells and enhance the shrinking and expansion of soil organic matter causing fragmentation and more surface exposure to chemical and biological activity. All the above processes will favor the release of SON. Low temperatures, on the other hand, decrease the rates of all chemical and biological reactions that generate SON. Freezing may also partially sterilize the system and thus, reduce the number of microorganisms responsible for decomposing organic matter.

Flooding appears to promote the production of soluble organic matter (Wang and Bettany 1993). The smaller amount of SON leached from the flooded-freeze-thaw treatment, as compared to the flooded treatment, indicates a dominance of a depressing effect of the freeze-thaw treatment over the enhanced production and solubilization of soluble N-containing organic substances that usually occurs under flooding conditions.

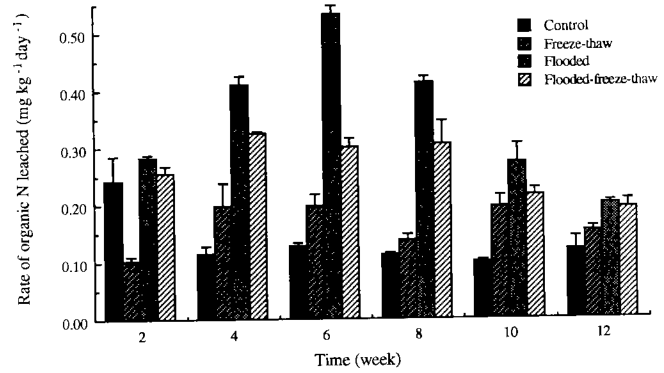

Fig. 1. Influence of freeze-thaw and flooding treatments on the average rate of organic N leached following each two-week incubation period. Error bars shown are standard errors of the means.

The dynamics of SON release are illustrated in the biweekly data (Fig. 1). The rate of leaching of SON from the freeze-thaw treatment was lower than the control in the first two weeks, but significantly higher ($P < 0.05$) later in the incubation period. This suggested that in the long-term freeze-thaw conditions would increase the leaching of SON from the unflooded soils. It may be that repeated freeze-thaw actions are required before significant fragmentation of organic matter occurs in this soil. The improvement of the soil structure in the later incubation period, if any, may also have a bearing on the explanation for the increased rate. It is known that freeze-thaw cycles affect soil structure by promoting the formation of aggregates, which in turn alters soil microbial population and composition.

The rates of leaching of SON for the flooded systems (flooded and flooded-freeze-thaw treatments) were always larger than those for the unflooded systems (control and freeze-thaw treatments). During the incubation period, an initial increase and then decrease in the rate of leaching of SON was observed for the flooded treatment. The maximum rate of SON leached (0.53 mg N kg^{-1} d^{-1}) for the flooded treatment occurred in the third incubation period (4–6 wk). A substantial difference between redox potentials of the flooded (344 to −46 mV) and unflooded systems (355 to 301 mV) was observed during the incubation period (Fig. 2). The rapid drop in the redox potential of the flooded system from 131 to 42 mV occurred in the third incubation period and coincided with the maximum rate of SON leaching. In a previous study this period was also coupled with a maximum release of soluble organic matter and highest CO_2 evolution (Wang and Bettany 1993). Thus, the maximum rate of SON leached may be attributed to the maximum leaching of soluble organic matter coupled with high microbial activity.

The higher concentration of SON in the leachates from the flooded soils compared to other treatments may be

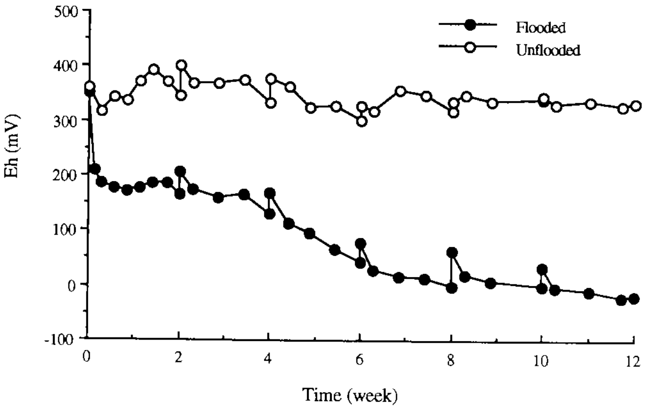

Fig. 2. Redox potentials of the flooded and unflooded soils immediately before and after leaching, and during the incubation period.

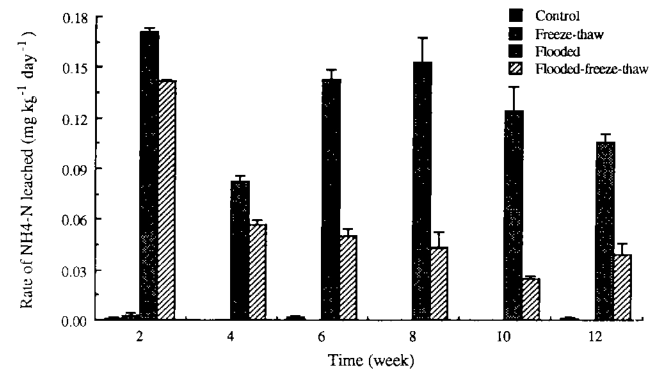

Fig. 3. Influence of freeze-thaw and flooding treatments on the average rate of NH_4-N leached following each two-week incubation period. Error bars shown are standard errors of the means.

also attributed to the lower soil Eh through its effect on reducing N mineralization. This is illustrated (Table 3) by the lower amounts of inorganic N and higher amounts of organic N leached from the flooded (11.8 and 29.6 mg kg^{-1}, respectively) compared with the control (97.94 and 11.4 mg kg^{-1}, respectively). Other workers (Smith et al. 1980; Beauchamp et al. 1986), have suggested that SON was easily mineralized in soils. Flooding, however, may promote the opposite result in that anaerobic deamination is much slower than aerobic deamination (Ponnamperuma 1972).

The cumulative loss of the total leachable N (sum of organic and inorganic N) for the treatments decreased in the order of control > freeze-thaw > flooded > flooded-freeze-thaw throughout the study (Table 3). However, soluble organic N as a percentage of the total leachable N showed the reverse trend, i.e., control (10.5%) < freeze-thaw (18.0%) < flooded (71.5%) < flooded-freeze-thaw (77.4%). The percentage value for the control was within the range of 9–12% reported by Beauchamp et al. (1986) for soils under similar conditions. The higher percentage of organic N in the total leachable N for the flooded compared to other systems was not only due to the larger amounts of leachable SON but also due to smaller quantities of inorganic N (Table 3). Therefore, a major pathway of potential leaching loss of N from flooded systems may be through organic N leaching.

Leaching of Inorganic Nitrogen

The leaching loss of NH_4-N among the treatments was in the order of flooded > flooded-freeze-thaw > control = freeze-thaw (Table 3). The rate of leaching of NH_4-N from the flooded treatment decreased initially in the second incubation period (2–4 wk), increased in the third and fourth incubation periods and then decreased again (Fig. 3). For the flooded-freeze-thaw treatment, a consistent decrease in the rate of leaching of NH_4-N was observed. There was virtually no accumulation of NH_4-N or change in the rate of leaching of NH_4-N for the unflooded systems during the incubation period. The lower amounts of NH_4-N in the flooded-freeze-thaw compared to flooded treatments again was probably due to the depressing effect of low temperatures on the rates of biological and biochemical reactions. This effect on ammonification in unflooded soils was well observed in other work (Dorland and Beauchamp 1990). The observation may also be due to a partial sterilizing of the microbial population that was responsible for N mineralization. The rates of CO_2 evolution from the freeze-thaw treated soils have been shown to be always lower than those for the treatments without freeze-thaw (Wang and Bettany 1993), indicating that the activity of soil microorganisms did not recover during the freeze-thaw cycle. The change in rate of leaching of NH_4-N from the flooded treatment did not show a consistent trend. The reason is not clear. Under flooding conditions, the amount of NH_4-N leached was about one-third of organic N leached. However, it constituted 17.4–26.3% of the total leachable N. Hence, NH_4-N may be an important component of the N lost by leaching from flooded systems.

In contrast to NH_4-N, leaching of the oxidized nitrogen (NO_3-N and NO_2-N) was the dominant process of N loss from the unflooded systems, accounting for 81.8–89.8% of the total leachable N (Table 3). The total loss of NO_3-N+NO_2-N from the treatments decreased in the order of control > freeze-thaw > flooded = flooded-freeze-thaw. In the flooded systems, the leaching loss of NO_3-N and NO_2-N was close to zero throughout the entire incubation period (Fig. 4), indicating that nitrification was inhibited. The rate of leaching of nitrate and nitrite from the freeze-thaw treatment was consistently less than that for the control during the incubation period (Fig. 4), indicating depressed nitrification probably due to the low temperature effect on the nitrifiers. Temperature has been shown to be one of the most influential factors affecting nitrification; below 5°C very little NO_3-N will be formed (Stevenson 1986). As suggested previously, the effect of partial sterilizing on the microorganisms involved in the nitrification may also have acted in concert with the low temperature effect.

The type and amount of inorganic N leached was clearly dependent on the Eh of the system. The amount of NH_4^+ in the leachates was low when the Eh was above 300 mV,

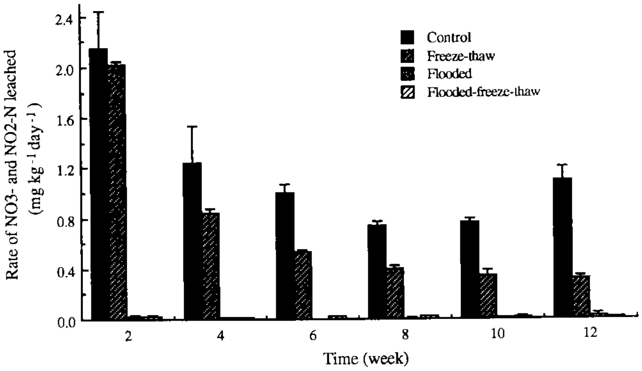

Fig. 4. Influence of freeze-thaw and flooding treatments on the average rate of NO_3- and NO_2-N leached following each two-week incubation period. Error bars shown are standard errors of the means.

i.e. in the unflooded systems. In contrast, there was virtually no $NO_3^- + NO_2^-$ present in the flooded systems as the Eh of the systems rapidly decreased below 200 mV (Figs. 2 and 4). Other supporting work on the sequential reduction of inorganic nitrogen showed that when Eh of a soil was lower than 200 mV, NH_4-N replaced NO_3-N completely (Patrick and Jugsujinda 1992). Dentrification may also have played a role in reducing the amount of nitrate and nitrite in the flooded systems.

SUMMARY AND CONCLUSIONS

Natural climatic events, such as flooding and freeze-thaw, may affect the quantity and quality of nitrogen leached from usually well-drained soils. The total leaching loss of N was less under both flooding and freeze-thaw conditions. The losses of SON and NH_4-N were enhanced by flooding. Soluble organic N was the main form of N in the leachates from the flooded treatments. In contrast, $NO_3^- + NO_2^-$ dominated the total leachable N in the unflooded treatments. In the short term, superimposing freeze-thaw conditions on the flooded and unflooded soils mainly reduced the amount of various nitrogen species in the leachates. A longer-term effect of freeze-thaw in the unflooded soil may be to increase the quantity of SON through the gradual breakdown of organic matter.

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