

Organic Chemicals in the Environment

Mineralization of Norflurazon in a Cranberry Bog Soil: Laboratory Evaluations of Management Practices

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ABSTRACT

Norflurazon (4-chloro-5-(methylamino)-2-(α,α,α -trifluoro-*m*-tolyl-3(2*H*)-pyridazinone) is a pre-emergent herbicide used in cranberry (*Vaccinium macrocarpon* Ait.) cultivation to control annual grasses, sedges, and broadleaf weeds. Cranberries are an economically important crop in New England, Wisconsin, and other parts of the northern USA. The biodegradation of norflurazon in the high organic matter, acidic soil characteristic of cranberry bogs has been shown to proceed slowly (Savin and Amador, 1998). The potential effects of cranberry cultivation practices—soil moisture control, fertilization, sand addition, and herbicide application rate—on mineralization of norflurazon in a bog soil were evaluated in a laboratory study. Optimal soil moisture for norflurazon mineralization was between 80 and 90% of water-holding capacity (WHC) in soil from the Oi and A horizons. Saturating the soil reduced the rate of norflurazon mineralization significantly. By contrast, soil respiration was maximal at 25% of WHC in both horizons. Addition of inorganic P increased soil respiration, but did not affect norflurazon mineralization significantly. Addition of inorganic N plus P increased soil respiration in the A, but not Oi, horizon and significantly decreased norflurazon mineralization in the Oi horizon. Sand addition had no significant effect on norflurazon mineralization. Mineralization was affected by herbicide application rate, with the rate of mineralization increasing proportionally with increasing concentration from 0.75 to 7.5 mg norflurazon kg⁻¹ soil. The mineralization of ¹⁴C-norflurazon was slow for all of the agronomic practices evaluated, indicating that the potential for norflurazon to accumulate in cranberry bog soils may be high.

THE fate of the pre-emergent herbicide norflurazon has been evaluated in mineral soils used to grow crops such as citrus, wheat (*Triticum aestivum* L.), and soybean [*Glycine max* (L.) Merr.] (Alva and Singh, 1990; Hubbs and Lavy, 1990; Lo and Merkle, 1984; Rahn and Zimdahl, 1973; Schroeder and Banks, 1986a,b; Singh et al., 1985; Southwick et al., 1993a, b). However, little information exists on either the fate of norflurazon (Savin and Amador, 1998) or the effects of agronomic practices on the fate of norflurazon in the acidic peat soils frequently used in cranberry production, even though norflurazon is used in cranberry cultivation to control annual grasses, sedges, and broadleaf weeds (WSSA Herbicide Handbook Committee, 1983).

Cranberry cultivation requires modification of existing peatlands through flood management, fertilization, and sand additions, as well as maintaining acidic conditions, with soil pH ideally between 3.8 and 5.5 (Deubert and Caruso, 1989). Fertilizers containing N and P are applied early in the growing season (mid-

May) to encourage higher vegetative growth, and/or in the summer (early August) to enhance fruit growth (University of Massachusetts Cranberry Experiment Station, 1986a). Cranberry bogs are flooded to aid harvesting in the autumn and prevent desiccation in the winter. Bog flooding is conducted periodically throughout the year to control insect damage and for frost protection (Deubert and Caruso, 1989). A 2.5-cm layer of sand is added every other winter onto the ice of flooded cranberry bogs which then settles into the soil as the ice melts in the spring (Deubert and Caruso, 1989). Sand is added to improve soil texture and soil aeration and to facilitate drainage (Deubert and Caruso, 1989). Norflurazon is applied at rates of 4.5 to 9 kg ha⁻¹ to cranberry bog soil between March and mid-April and/or in the fall following cranberry harvest (Deubert and Caruso, 1989).

Cranberry bog soils differ from mineral soils in their extreme acidity, low nutrient content, high water retention capacity, and high organic matter (OM) content (Mitsch and Gosselink, 1993). Norflurazon is sorbed to soil OM and is not highly mobile even in low OM mineral soils (Alva and Singh, 1990; Singh et al., 1985; Savin and Amador, 1998). Microbial metabolism of norflurazon may be an important process affecting the fate of norflurazon in acidic, high OM soil. Results of a previous study indicate that degradation of norflurazon in a cranberry bog soil is primarily biologically mediated (Savin and Amador, 1998). Experiments using ¹⁴C-norflurazon applied at a rate of 4.0 mg norflurazon kg⁻¹ soil to Oi and A horizon soil showed that about 5% of norflurazon was converted to ¹⁴CO₂ during a 6-wk incubation, with 1 to 2% of radioactivity incorporated into microbial biomass following incubation for 1 mo, and 10 and 18% of initial radioactivity was associated with a more polar metabolite in Oi and A horizon soil, respectively, after incubation for 9 wk, with the remaining radioactivity associated with norflurazon (Savin and Amador, 1998).

Microbial mineralization may be one of the few, if not the only, soil processes by which norflurazon is completely detoxified in acidic peat soil. Agronomic practices generally alter soil physical and chemical properties and can affect the composition and activities of microbial communities, potentially affecting the microbial degradation of norflurazon in soil. As such, the principal objective of this study was to assess the effects of cranberry cultivation practices on microbial mineralization of norflurazon in the surface Oi and A horizons of a cranberry bog soil. Specifically, we evaluated the effects of varying moisture content, fertilizer amend-

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ments, sand addition, and herbicide application rate on norflurazon mineralization.

MATERIALS AND METHODS

Soil Sampling

Soil samples were collected from a small (~200 m²), privately-owned, constructed cranberry bog in Richmond, RI, and stored for up to 14 d in plastic bags at room temperature in the dark. The bog is level and drainage is not controlled. The soil is a poorly drained sandy loam, classified as a sandy, mixed, mesic Aeric Haplaquept from the Walpole soil series (Soil Survey Staff, 1981). Soil samples were obtained from the top, fibric organic layer (Oi horizon) and the surface soil layer (A horizon).

Soil Properties

Soil pH was measured using a 1:2 (wt/wt) soil/water ratio for A horizon soil and a 1:5 soil/water ratio for Oi horizon soil (Hendershot et al., 1993). The organic matter content of the soil was determined by loss-on-ignition at 550°C for 4 h (Karam, 1993). The bulk density of the soil was determined gravimetrically by oven drying a known volume of soil at 105°C overnight to determine the mass of solids. Soil water content was determined gravimetrically after oven drying a known mass of moist soil at 105°C overnight. The WHC of the soil was determined by placing saturated soil in a Büchner funnel, allowing it to drain freely for 2 d, and subsequently determining the gravimetric water content of the soil.

The microbial biomass C content of the soil was determined by the fumigation-extraction method, using a correction factor of 2.64 (Vance et al., 1987). The amount of organic C in the extract was measured by titration (Anderson and Ingram, 1993). The total N content of the soil was determined by Kjeldahl digestion and total P by inductively coupled plasma spectrophotometry following magnesium nitrate ashing, at the Nutrient Analysis Laboratories of Cornell University (Ithaca, NY). Water-extractable PO₄³⁻ (Olsen and Sommers, 1982) and KCl-extractable NH₄⁺ and NO₃⁻ (Keeney and Nelson, 1982) were analyzed colorimetrically using an Alpkem Rapid Flow Analyzer (RFA-300, Alpkem Corp., Clackamas, OR) (Anonymous, 1986). Soil properties are shown in Table 1.

Table 1. Selected physical, chemical, and microbiological properties of the two soil horizons used in this study. Values are means (*n* = 3).

Soil property	Soil horizon	
	Oi	A
Depth, cm	0-7	7-24
Texture	NA†	Fine sandy loam
Color	NA	10YR 2/1
Structure	NA	Subangular blocky
Consistency	NA	Friable
pH	4.7	3.7
Bulk density, g cm ⁻³	ND‡	0.96
Water-holding capacity, %	630	127
% OM content	50.5	12.3
Microbial biomass, mg C kg ⁻¹ soil	1800	510
Organic C, g kg ⁻¹ soil§	300	72
Total N, g N kg ⁻¹ soil	13.2	2.7
Total P, g P kg ⁻¹ soil	0.7	0.1
C/N	23:1	27:1
C/P	424:1	724:1
NH ₄ ⁺ -N, (mg N kg ⁻¹ soil)	217	51
NO ₃ ⁻ -N (mg N kg ⁻¹ soil)	0.94	0.14
PO ₄ ³⁻ -P (mg P kg ⁻¹ soil)	0.79	0.10

† Not applicable.

‡ Not determined.

§ The fraction of OM divided by 1.7 (Nelson and Sommers, 1982).

Norflurazon

Unlabeled and ¹⁴C-pyridazinyl-ring-labeled norflurazon (Fig. 1) were a gift from Sandoz Agro, Inc./Sandoz Crop Protection Corp. (Des Plaines, IL). The specific activity of the labeled norflurazon was 41.1 mCi mmol⁻¹ and it had a radiopurity of 99.8% as determined by the manufacturer. The unlabeled compound was 99.49% pure as determined by the manufacturer.

Mineralization of Carbon-14 Norflurazon

Soil (20–30 g) was placed in a biometer flask (Bellco Glass Inc., Vineland, NJ) (Bartha and Pramer, 1965) and an aqueous solution containing a known concentration of unlabeled and ¹⁴C-pyridazinyl-ring-labeled norflurazon (~0.2 μCi per flask) was added. The flasks containing the amended soil were incubated statically in the dark at 25°C in a gravity-convection incubator. Evolved ¹⁴CO₂ was trapped in the side-arm of the biometer flask in 5 mL of 1 M NaOH solution. Aliquots (1.0 mL) of NaOH solution containing the ¹⁴CO₂ were mixed with scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, GA) and the radioactivity of the solution determined by liquid scintillation counting after a 24-h equilibration period (LKB Wallac 1209 Rackbeta Liquid Scintillation Counter, Pharmacia LKB, Gaithersburg, MD).

Soil Respiration

Soil respiration was measured by gas chromatographic (GC) analysis of CO₂ evolved following static incubation of soil samples at 25°C (Amador and Jones, 1993). A known amount of soil (~2 g) was placed in a 20-mL glass serum vial, appropriate amendments made, and the vial sealed using a rubber septum and an aluminum crimp collar. A GC fitted with a thermal conductivity detector was used for CO₂ analysis with hydrogen as a carrier gas. Periodically, a sample of headspace gas (1.0 mL) was removed by displacement using an automated headspace sampler (Tekmar 7000, Cincinnati, OH) and injected into the GC. A Porapak T column (80/100 mesh, 10 ft) was used to separate CO₂ at 60°C. Standards containing a known concentration of CO₂ were analyzed in triplicate with each set of soil samples and used to convert peak areas to concentration of CO₂. After analysis, the headspace of the sample vials was evacuated and replaced with fresh air at least five times before resuming incubation.

Soil Moisture

To determine the effects of soil moisture on mineralization of norflurazon and soil respiration, the moisture content of air-dried soil was adjusted with deionized, distilled (DI) water to the desired percentage of WHC and immediately measured for CO₂ evolution.

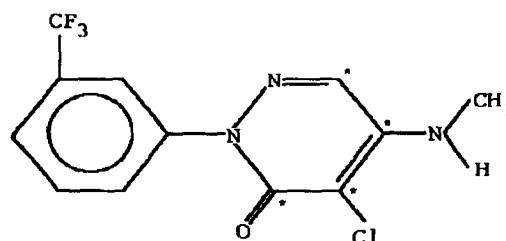


Fig. 1. Structure of norflurazon. Radiolabeled carbons are marked with an asterisk.

Nutrient Additions

Inorganic N (as NH_4Cl) and inorganic P (as Na_2HPO_4) were added to soil at a rate of 2 g N or P kg^{-1} soil. Fertilizers with ratios of 1:2 N/P are recommended with seasonal N applications of 8.9 to 53.5 kg ha^{-1} , depending on a particular crop's needs (DeMoranville, 1993). These rates convert to doses of approximately 0.3 to 1.7 g N kg^{-1} dry soil in the biometer flasks. Effects of nutrient additions on mineralization of norflurazon and endogenous respiration were assessed in soil at 75 to 85% of WHC.

Sand Addition

Sand was mixed into A horizon soil at a 1:1 (wt/wt) ratio and mineralization of norflurazon compared to that in soil without sand. Initial soil moisture content in both treatments was approximately 70% of WHC. No attempt was made to keep water content constant following sand addition.

Data Analysis

Linear regression was used to compute initial norflurazon mineralization rates (Day 0–10, $r^2 \geq 0.80$). Mean values ($n = 3$ for all treatments) of rate and extent of mineralization were compared using a one-way analysis of variance. Multiple comparison testing to assess significant differences between groups was done using the Bonferroni *t*-test. All tests were evaluated at the 95% confidence level.

RESULTS AND DISCUSSION

Soil Moisture Content

If substrate diffusion and O_2 diffusion limit aerobic microbial activity, this activity should be maximal at a water content where substrate and O_2 diffusion are equally limiting to microbial populations (Skopp et al., 1990). We hypothesized that soil water content should affect norflurazon mineralization such that as soil water content increases, the rate of mineralization will increase up to a critical value, beyond which mineralization rate will decrease with further increases in water content. To test this hypothesis, the mineralization of 4.0 mg herbicide kg^{-1} soil in Oi and A horizon soil held at different water contents was evaluated. Initial rate and total cumulative value of $^{14}\text{CO}_2$ evolution were highest at water contents of 80 to 90% of WHC for both soils, with norflurazon mineralization significantly lower under flooded and low soil moisture regimes (Fig. 2). Mineralization rate in soil at the lowest water content was comparable to abiotic mineralization (Savin and Amador, 1998). In contrast, soil respiration was highest at 25% of WHC for the Oi and A horizon soil and decreased with increasing water content (Fig. 3). The initial high rate of soil respiration at the low water contents may have been the result of microbial acclimation not seen at other water contents.

Our results indicated that a relatively high water content in both the Oi and A horizons was optimal for norflurazon mineralization. It is generally considered that maximal microbial activity, and consequently biodegradation of natural and xenobiotic compounds, will occur at approximately 60% of WHC or water-filled pore space (Greaves and Carter, 1920; Foster and

McKercher, 1973; Linn and Doran, 1984; Skopp et al., 1990; Amador and Jones, 1997). Below the optimal water content, substrate availability limits microbial activity; above the optimal water content, O_2 diffusion becomes limiting (Linn and Doran, 1984; Skopp et al., 1990). Given the highly porous nature of these soils, high water contents may be necessary for desorption and adequate substrate diffusion and accessibility to the microorganisms, with O_2 limitations becoming apparent only under flooded conditions. Previously, we found that norflurazon is highly sorbed to the soil (Savin and Amador, 1998) and the rate of desorption may limit degradation. Also, addition of cycloheximide to soil reduced norflurazon mineralization to less than half the rate and extent of that found in soil with no inhibitor added, suggesting that fungi are important contributors to norflurazon mineralization in the surface horizons of the bog (Savin and Amador, 1998). Fungi, as well as aerobic bacteria, are less likely to meet their metabolic demand under the anaerobic conditions likely to develop in saturated soil (Alexander, 1977), possibly explaining the marked decrease in mineralization following flooding.

In contrast, soil respiration appeared to be limited

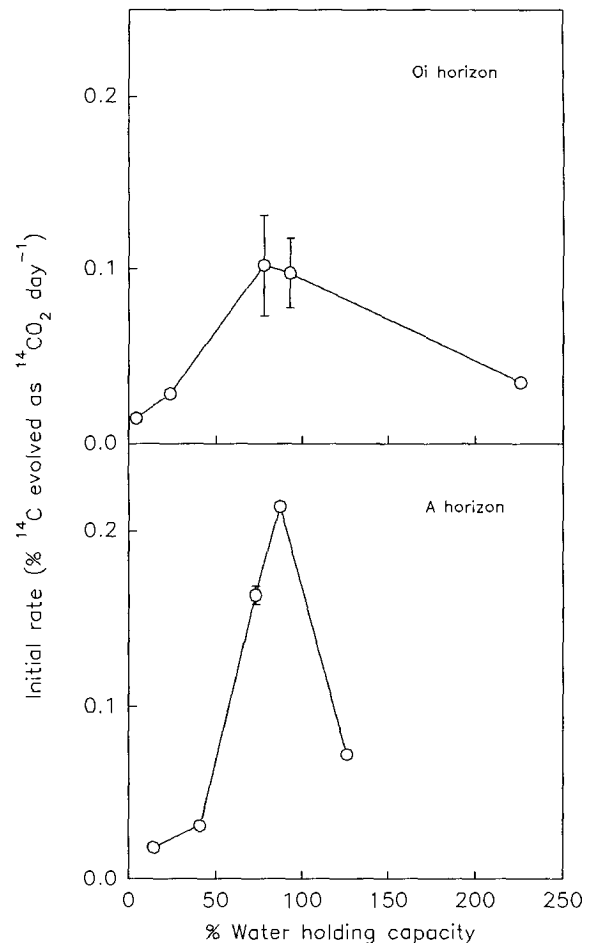


Fig. 2. Initial mineralization rate (0–10 d) of 4.0 mg norflurazon kg^{-1} soil in Oi and A horizon soil as a function of soil moisture content. Error bars represent 1 SD and are not shown if smaller than the datum point.

by O₂ diffusion, as indicated by a lower optimal water content (Fig. 3). The relative rates of respiration and norflurazon mineralization in Oi and A horizon soil as a function of soil water content suggest that the microbial populations involved in norflurazon mineralization are affected differently by changes in soil water status from the populations involved in OM mineralization (Fig. 2 and 3). Furthermore, these differences are consistent in both soil horizons. The differential response to soil moisture may indicate different tolerances to O₂ stress among mineralizing populations and/or spatial differences in degradation, with OM mineralization occurring in the smaller pores—likely to be filled at lower water contents—and norflurazon mineralization occurring in the larger pores, which are filled only at higher water contents.

Nutrient Additions

The C/P ratio of the bog soil (Table 1) is considerably higher than that of soil microorganisms (20:1–30:1), while the C/N ratio is closer to that of soil microflora (5:1–15:1) (Alexander, 1977; Paul and Clark, 1989). Phosphorus is highly immobilized in acidic soil, where

it tends to precipitate and bind to ferric and aluminum oxides and hydroxides. Inorganic P is released by slow decay of organic matter in acidic bog soils (University of Massachusetts Cranberry Experiment Station, 1986b) and therefore it is often a limiting nutrient for plant productivity and microbial activity. Hence, we hypothesized that addition of inorganic P to the bog soil would increase microbial degradation of norflurazon as well as respiration, but addition of N would have no effect.

We tested this hypothesis by determining the effects of PO₄³⁻, NH₄⁺, and combined PO₄³⁻ and NH₄⁺ amendments on soil respiration. Soil respiration was enhanced by P amendments in both Oi and A horizons, whereas combined N and P amendments increased respiration in the A, but not the Oi, horizon (Fig. 4). Addition of N alone depressed soil respiration in the Oi horizon, but had no apparent effect on respiration in the A horizon. It appears that P amendments removed P limitations to mineralizing populations in both soil horizons, while N

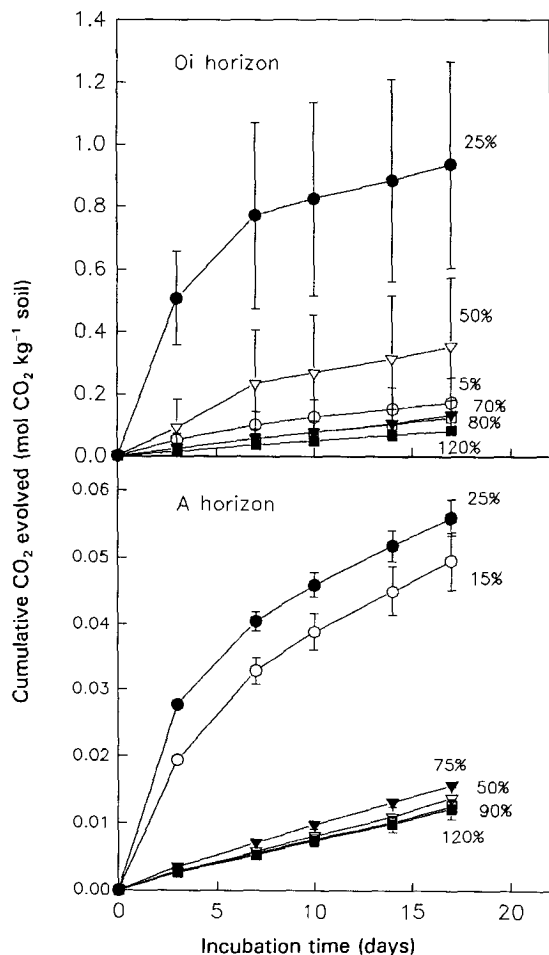


Fig. 3. Effect of soil moisture content on soil respiration in Oi and A horizon soil. Numbers next to curves represent percent of water-holding capacity. Error bars represent 1 SD and are not shown if smaller than the datum point.

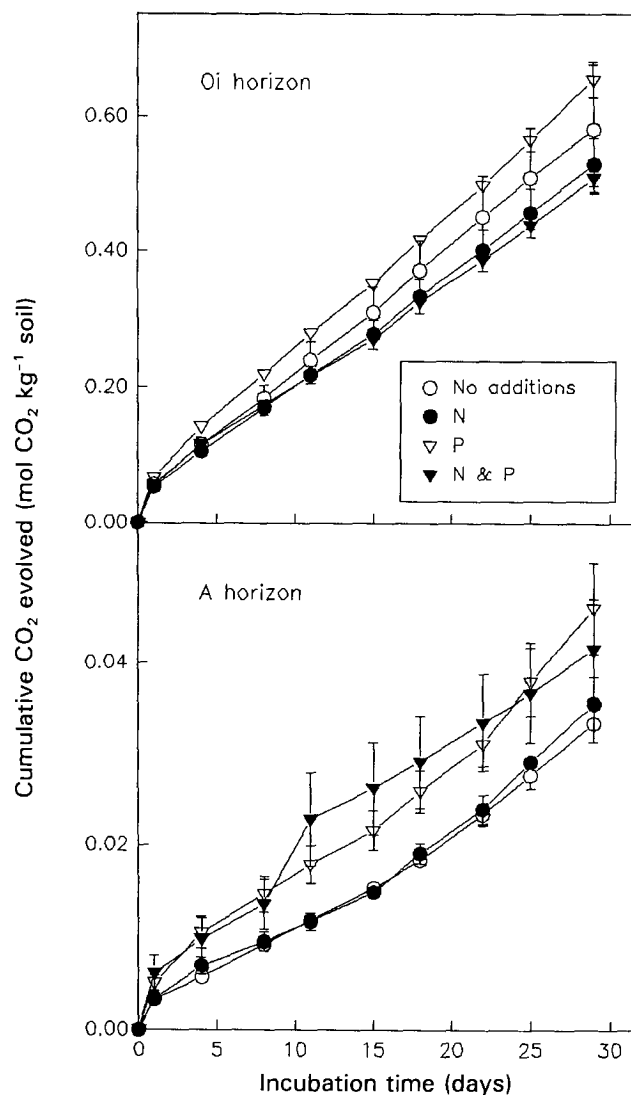


Fig. 4. Effects of inorganic nutrient amendments on soil respiration in Oi and A horizon soil. Added nutrient concentrations were 2 g N or P kg⁻¹ soil. Error bars represent 1 SD and are not shown if smaller than the datum point.

plus P amendments removed limitations of microbial populations specific to the A horizon. Potassium sulfate extracts of Oi horizon soil averaged 660 mg C kg^{-1} soil, whereas in A horizon soil, extracts averaged 200 mg C kg^{-1} soil. Combined with the high levels of nutrient additions, the lower respiration observed in N, and N plus P treatments in the Oi horizon may result from higher C immobilization. Similarly, Amador and Jones (1993) found that addition of PO_4^{3-} to neutral peat soil from the Everglades with initial C/P and C/N ratios close to those of soil in our study increased respiration, while NH_4^+ amendments decreased respiration, indicating immobilization of C resulting from excess available N (Amador and Jones, 1993).

Based on these results, the effects of P, and combined N and P amendments on norflurazon mineralization were evaluated. Contrary to our hypothesis, mineralization of norflurazon was not significantly enhanced by the addition of PO_4^{3-} in either horizon, and combined addition of N and P inhibited the extent of herbicide mineralization in Oi horizon soil significantly (Fig. 5). Nitrogen and P availability to the microbial populations in the bog soil does not appear to limit norflurazon mineralization. It should be noted that norflurazon mineralization in the control treatments in this experiment was lower than in the controls of other experiments. This is not unexpected, given the inherent variability of soils in terms of chemical, physical, and biological properties (e.g., Wollum, 1994).

Sand Addition

Addition of sand to A horizon soil did not affect the rate or extent of norflurazon mineralization significantly (data not shown). If sand increases aeration in the soil

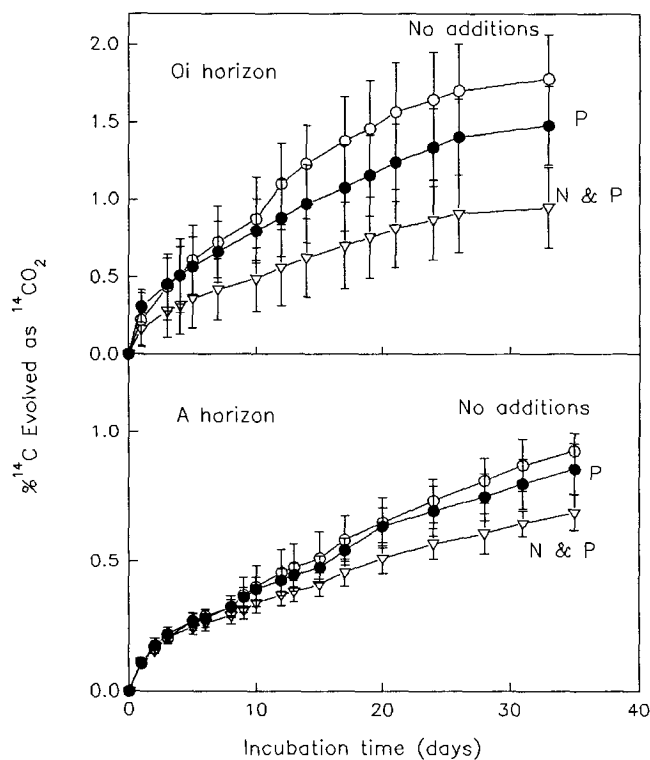


Fig. 5. Effects of nutrient amendments on mineralization of $4.0 \text{ mg norflurazon kg}^{-1}$ soil in Oi and A horizon soil. Error bars represent 1 SD.

(Deubert and Caruso, 1989), these results suggest that substrate diffusion may be more critical in limiting norflurazon mineralization than O_2 diffusion. Increased norflurazon mineralization at high, but not saturated, soil water contents (Fig. 2) supports this interpretation.

Herbicide Application Rate

The mineralization rate of organic compounds generally increases as concentration of the compound in the soil increases (Alexander, 1985). We hypothesized that the rate of norflurazon mineralization would increase with increasing herbicide application rate. Mineralization was assessed at 0.75 , 4.0 , and $7.5 \text{ mg norflurazon kg}^{-1}$ A horizon soil (approximately 0.05 , 0.23 , and 0.45 kg ha^{-1}). The initial (0 – 10 d) mineralization rate of norflurazon was 1 , 9 , and $15 \text{ } \mu\text{g kg}^{-1} \text{ soil d}^{-1}$ (Fig. 6, inset). Although the highest percentage of norflurazon evolved as $^{14}\text{CO}_2$ was observed at the $4.0 \text{ mg norflurazon kg}^{-1}$ soil (Fig. 6), when converted to concentration, the total amount of norflurazon mineralized to $^{14}\text{CO}_2$ after 7 wk was 30 , 210 , and $370 \text{ } \mu\text{g kg}^{-1}$ soil, in treatments with initial concentrations of 0.75 , 4.0 , and $7.5 \text{ mg norflurazon kg}^{-1}$ soil, respectively. When converted to amount of norflurazon mineralized, the rate of mineralization increases linearly with application rate (Fig. 6, inset).

The linear increase in initial mineralization rate with increasing norflurazon concentration indicates that the mineralization rate is limited by substrate concentration at agronomically relevant norflurazon application rates. The norflurazon mineralization curves had similar shapes regardless of herbicide concentration (Fig. 6), suggesting that mineralization kinetics were unchanged over the concentration range evaluated. Furthermore, the concave-downwards shape of mineralization curves

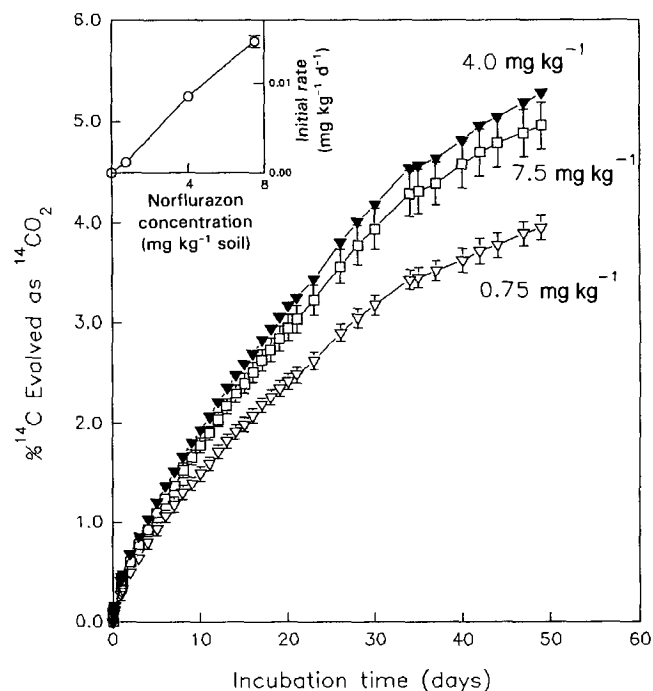


Fig. 6. Effect of initial substrate concentration on norflurazon mineralization in A horizon soil. Numbers next to the curves indicate norflurazon concentration in mg kg^{-1} soil. Error bars represent 1 SD and are not shown if smaller than the datum point.

at all substrate concentrations indicates that mineralizing populations did not grow at the expense of norflurazon. Growth-related biodegradation kinetics are usually associated with concave-upwards mineralization curves (Alexander and Scow, 1989).

CONCLUSIONS

Neither nutrient amendments nor sand addition significantly enhanced norflurazon mineralization in either the Oi or A horizons of the bog soil. Mineralization rate increased proportionally with increasing norflurazon application rate. It appears that manipulation of cranberry cultivation practices to enhance norflurazon mineralization may be limited to the control of soil moisture. Norflurazon mineralization is greatest at high water contents (about 80–90% of WHC), although it is inhibited by flooding. Water management practices such as drainage following short-duration floods may maintain a water content sufficiently high to maximize norflurazon degradation. The high OM content of bog soil means it has a high water retention capacity, increasing the potential for desorption and substrate diffusion and contact with the microorganisms involved in norflurazon mineralization.

Given that the mineralization of ¹⁴C-norflurazon was slow for all of the agronomic practices evaluated, the potential for norflurazon to persist in cranberry bog soils may be high. Persistence may also lead to continuous input of norflurazon in bog drainage waters. Others (Murphy and Shaw, 1997) have shown that norflurazon can serve as a continuing contaminant in runoff from mineral soils used in cotton cultivation.

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