

DIVISION S-3—NOTES

HYDROLOGIC TRACER EFFECTS ON SOIL MICROBIAL ACTIVITIES

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Abstract

While much research has shown that the commonly used hydrologic tracers Br^- , Cl^- , and pentafluorobenzoic acid (PFBA) are not affected by soil microbial processes, much less work has gone into analysis of the effects of these tracers on microbial activity. In this study, we analyzed the effects of Br^- , Cl^- , and PFBA at 100 mg kg^{-1} concentration on soil respiration, denitrification, N mineralization, and nitrification in a forest soil under laboratory conditions. Chloride and Br^- inhibited all activities other than denitrification and PFBA stimulated respiration and denitrification. Mechanisms for the observed effects may be related to the effects of these anion tracers on soil solution cation activities and microbial membrane processes. The results suggest that effects on microbial activities should be considered when tracers are used in studies of the fate and transport of pollutants that are affected by microbial processes.

A VARIETY OF COMPOUNDS are used to trace the movement of water through environmental media. While a large body of work has gone into determining that these compounds are not degraded by microbial processes, much less work has gone into analysis of the effects of hydrologic tracers on microbial activity. These effects could be important when tracers are used in studies of the fate and transport of pollutants in the environment. In such studies, it is important to determine if tracer compounds affect microbial processes that influence pollutant dynamics.

Tracer effects on soil microbial processes could be important in several contexts. Small anion tracers such as Cl^- or Br^- are often added as Na, K, Ca, or Li salts. High salt concentrations have been found to inhibit general microbial activity and Cl^- has been found to be a potent inhibitor of nitrification, the oxidation of NH_4^+ to NO_3^- , (Hahn et al., 1942; Heilman, 1975; Johnson and Guenzi, 1963; Agarwal et al., 1971; Roseberg et al., 1986), and plant growth (Tisdale et al., 1985, p. 395). Organic tracers (e.g., fluorobenzoates) may be susceptible to microbial degradation under anaerobic conditions (Suffita et al., 1982; Bowman and Gibbens, 1992) and can therefore stimulate microbial activity in anaerobic media. Effects of tracers on overall microbial activity will affect general degradation and cometabolism reactions of pollutants. Effects on specific activities such

as nitrification and denitrification (the conversion of NO_3^- to N gas) can be important to the fate of NO_3^- and organic pollutants such as halogenated aliphatic compounds that are degraded by specific microbial groups (Vanelli et al., 1990; Rasche et al., 1991).

In this study, we evaluated the effects of Cl^- , Br^- , and PFBA on soil respiration, denitrification, nitrification, and N mineralization in a forest soil under laboratory conditions. Our objective was to determine if any of these tracers had significant effects on microbial activity that should be considered when choosing tracers for studies of the fate and transport of pollutants in soils.

Methods

The soil used for these experiments is a Rainbow silt loam (coarse-loamy, mixed, mesic Aquic Dystrachrept, pH [measured in a 1:1 soil/water paste] ≈ 4.5) taken from an approximately 75-yr old oak (*Quercus* sp.) dominated forest in Kingston, RI. Sampling included both O and A horizons. Samples were sieved ($>4 \text{ mm}$) and were held at field moisture at 4°C between the time of sampling and analysis.

Soil respiration was assayed by placing 25-g soil samples in 0.946-L Mason jars with caps containing septa for headspace sampling. Triplicate samples were treated with 25 mL of distilled water (controls) or 25 mL of 100 mg L^{-1} solutions of KCl, KBr, or PFBA. The 100 mg L^{-1} solutions equal 2.82 mM Cl^- , 1.25 mM Br^- , and 0.47 mM PFBA . An additional set of samples was treated with 25 mL of a 1000 mg L^{-1} glucose solution (amended control) or 25 mL of a 1000 mg L^{-1} glucose solution containing 100 mg L^{-1} KCl, KBr, or PFBA. Glucose was added to relieve any energy limitation of heterotrophic microbes to reduce the potential for microbial degradation of PFBA. Samples were incubated for 10 d at room temperature. Gas samples were removed from the headspace of the jars every 2 d and were analyzed for CO_2 using an SRI (Redondo Beach, CA) 8610 gas chromatograph equipped with a methanizer (to convert CO_2 to methane) and a flame ionization detector.

Denitrification was measured in 25-g soil samples in 125-mL Erlenmeyer flasks. Triplicate samples were treated with 25 mL of a 100 mg N L^{-1} solution of KNO_3 (controls) or 25 mL of a 100 mg N L^{-1} solution of KNO_3 containing 100 mg L^{-1} KCl, KBr, or PFBA. Flasks were sealed with air-tight stoppers and were made anaerobic by repeated evacuation and flushing with O_2 -free gas. Acetylene (5 mL) was added to the headspace of the flasks to make N_2O rather than N_2 the end product of denitrification (Yoshinari and Knowles, 1976). Gas samples were removed from the headspace at 6.5, 13, and 24 h and analyzed for N_2O using a Tracor (Austin, TX) 540 gas chromatograph equipped with an electron capture detector.

Potential net N mineralization and nitrification were measured in 25-g soil samples in 125-mL Erlenmeyer flasks. Triplicate samples were treated with 25 mL of distilled water (controls) or 25 mL of 100 mg L^{-1} solutions of KCl, KBr, or PFBA. Samples were shaken for 24 h at 125 rpm on an orbital shaker and then filtered and analyzed for NH_4^+ and NO_3^- concentrations using an AlpKem (Clackamas, OR) RFA 300 continuous-flow analyzer. Potential net N mineralization was calculated as the accumulation of NH_4^+ plus NO_3^- , and

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net nitrification was calculated as the accumulation of NO_3^- during the 24-h incubation.

Results were analyzed by one-way analysis of variance with Fisher's protected least significant difference multiple comparisons test to compare different treatments (SAS Institute, 1985). Separate analyses were done for each time point, and for incubations with and without glucose in the respiration assays. Data were log-transformed before analysis where appropriate.

Results

Soil respiration at 2 d was lower ($P < 0.05$) in the presence of Cl^- , Br^- , and PFBA than in controls under unamended conditions, but the difference was significant ($P < 0.05$) across all points for Cl^- only (Fig. 1). In glucose-amended incubations, activity in all tracer treatments was higher relative to controls than in unamended incubations. In the glucose-amended incubations, respiration was higher ($P < 0.05$) in PFBA-treated samples than in controls or Cl^- -treated samples (Fig. 1). There was no difference in respiration between unamended and glucose-amended controls (Fig. 2).

Denitrification tended to be inhibited by Cl^- and stimulated by PFBA, but the differences were generally not

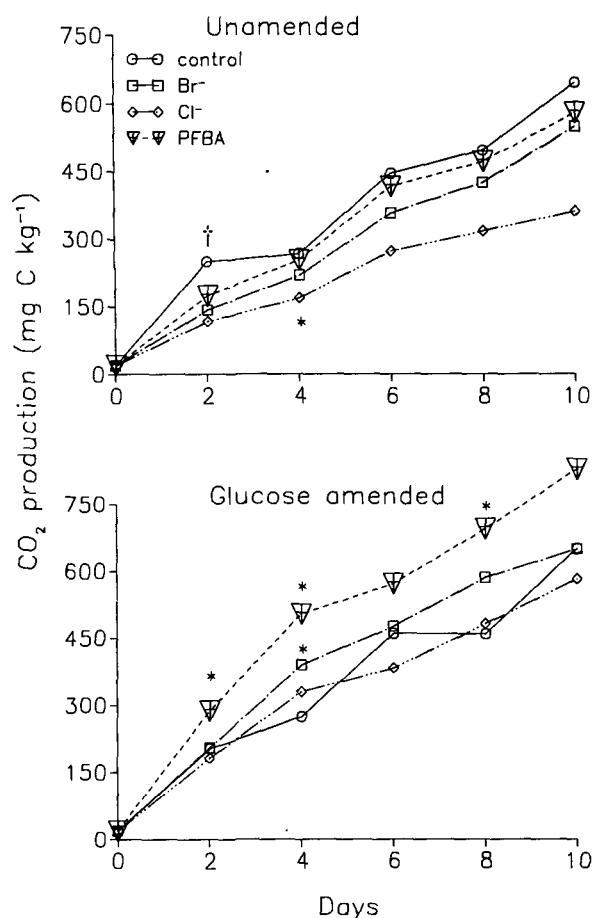


Fig. 1. Soil respiration in unamended and glucose-amended control and tracer-treated soils. Values are means, error bars are standard errors. *Significant ($P < 0.05$) difference between treated and control. †Control significantly ($P < 0.05$) higher than all treatments.

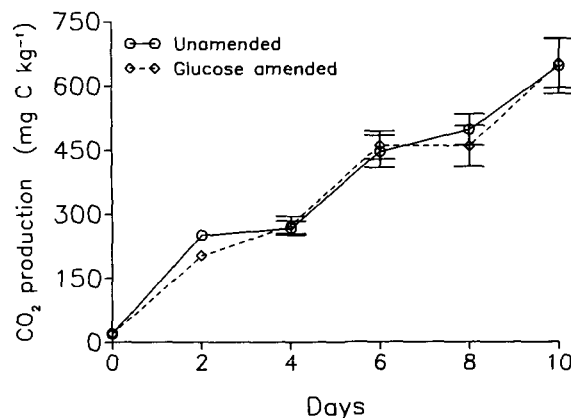


Fig. 2. Soil respiration in unamended and glucose-amended controls. Values are means with standard error bars.

significant ($P < 0.05$, Table 1). Potential net N mineralization and nitrification were inhibited ($P < 0.05$) by Br^- and Cl^- (Table 2).

Discussion

The results suggest that Cl^- , Br^- , and PFBA can affect microbial activity at concentrations commonly used in field hydrology studies. The Cl^- and Br^- concentrations that we used in these experiments were the same as those used in two of our field hydrology-microbiology studies (Simmons et al., 1992; Groffman et al., 1992; Nelson et al., 1995) where tracer solutions were introduced directly into groundwater. In such studies, it is often necessary to introduce high concentrations of tracers due to dilution and dispersion in the groundwater. Smith et al. (1992) reported Br^- concentrations as high as 250 mg L^{-1} in natural gradient tracer test studies of groundwater denitrification and CH_4 oxidation. Harvey et al. (1993) used 150 mg L^{-1} Br^- in studies of bacterial transport in groundwater. Gillham et al. (1990) used 47 mg L^{-1} Br^- in studies of groundwater denitrification. Bowman (1984) used 40 mg L^{-1} PFBA and Bowman and Gibbens (1992) used 150 mg L^{-1} PFBA in groundwater transport studies. Tracer concentrations are even higher in studies where tracer compounds are applied to surface soils. Owens and Edwards (1992) applied $168 \text{ kg Br}^- \text{ ha}^{-1}$, Jemison and Fox (1991) applied $100 \text{ kg Br}^- \text{ ha}^{-1}$, and Kung (1990) applied $112 \text{ kg Br}^- \text{ ha}^{-1}$ to surface soils. These applications probably produced Br^- concen-

Table 1. Denitrification in soils treated with 100 mg kg^{-1} of different tracers.

Time h	N_2O production			
	Control	Cl^-	Br^-	PFBA
6.5	18.9 (2.3)†a‡	25.3 (25.3)ab	20.2 (1.7)a	31.6 (3.1)b
13	32.7 (30.6)ab	9.2 (4.6)a	33.7 (9.1)ab	73.9 (14.2)b
24	227.9 (103)ab	146.3 (49.9)a	225.6 (22.7)ab	304.5 (17.2)b

† Values are means with standard errors in parentheses.

‡ Values followed by different superscripts within a row are significantly different at $P < 0.05$ in a one-way analysis of variance with a Fisher's protected LSD test.

Table 2. Potential net N mineralization and nitrification in soils treated with 100 mg kg⁻¹ of different tracers.

	Control	Br ⁻	Cl ⁻	PFBA
	mg N kg ⁻¹ d ⁻¹			
Mineralization	7.7 (1.5)†a‡	2.5 (0.6)b	1.9 (0.2)b	9.6 (0.6)a
Nitrification	3.2 (1.2)a	0.2 (0.01)b	0.2 (0.1)b	2.2 (1.5)a

† Values are means with standard errors in parentheses.

‡ Values followed by different superscripts within a row are significantly different at $P < 0.05$ in a one-way analysis of variance with a Fisher's protected LSD test.

trations well in excess of 100 mg L⁻¹ in surface soil water.

The magnitude of the effects of tracers on microbial activities observed in this study would be quite significant in field studies. Soil respiration, an index of total microbial activity, was reduced by approximately 50% over 2 d in the Cl⁻ and Br⁻ treatments (not glucose amended) and was stimulated by 25% in the PFBA treatment (glucose amended). Nitrification was <10% of the control and mineralization was <35% of the control in the Cl⁻ and Br⁻ treatments. These results suggest that tracers could affect both heterotrophic and chemoautotrophic processes in groundwater. It is clear that evaluation of the effects of tracers on microbial processes should be done, using realistic environmental media, tracer concentrations, and exposure times when tracers are used in any study where microbial processes are a factor.

It is important to note that the molarity of 100 mg L⁻¹ solutions differs from tracer to tracer. The molarity of the Cl⁻ solution was more than twice that of the Br⁻ solution, and more than five times that of the PFBA solution. The difference in molarity may partly explain why Cl⁻ had the most marked effects on microbial activity.

Several previous studies have also found Cl⁻ inhibition of nitrification. The lowest inhibiting concentrations in these studies ranged from 95 (Hahn et al., 1942) to near 500 mg L⁻¹ (Agarwal et al., 1971; Heilman, 1975; Roseberg et al., 1986). Agarwal et al. (1971) observed inhibition of soil respiration by Cl⁻ at 400 mg L⁻¹. Conversely, Johnson and Guenzi (1963) did not observe a significant inhibition of soil respiration by a range of Cl⁻ solutions of different osmotic tensions (from 0–3 MPa).

The mechanism by which Cl⁻ inhibits microbial activity is undetermined. The studies cited above determined that the effect is independent of solution osmotic strength and the cation associated with the Cl⁻ that is added. Roseberg et al. (1986) observed that inhibition of nitrification was increased at low pH and disappeared altogether at pH >6.0. Our data suggest that glucose additions relieve Cl⁻ inhibition of soil respiration but the mechanism for this relief is unclear. The glucose effect did not appear to be due to stimulation of microbial activity. Glucose-amended controls respired at the same rate as unamended controls, suggesting that microbes were not limited by C availability.

The effects of PFBA on microbial activity were surprising since this compound has been found to be resistant to microbial degradation (Bowman and Gibbens, 1992). It seems unlikely that PFBA stimulated soil respiration

by providing substrate to soil microbes since microbes were not C limited. Moreover, the fact that PFBA stimulation of respiration was only observed in glucose-amended incubations, and that PFBA also stimulated nitrification (an autotrophic process), is further evidence that PFBA effects on microbial activity are not related to microbial C dynamics.

The effects of these tracers on microbial processes may be caused by alteration of the chemical activities of different ions in the soil solution, which can affect membrane functions in microbes. Different anions have specific effects on the chemical activities (e.g., availability) of soil solution cations (Jakobsen, 1992). Excessive Cl⁻ additions have been found to adversely affect plant root function (Tisdale et al., 1985, p. 395; Jakobsen, 1992) and may also affect microbial activity. Different anionic tracers may affect the activity of NH₄⁺ in the soil solution, hindering or helping the ability of nitrifiers to take up their substrate. The different anionic tracers may also have differential effects on NO₃⁻ uptake by denitrifiers and on the general ability of heterotrophs to take up substrates to support respiration.

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