# SHORT COMMUNICATIONS

## Differences in Microbial Biomass, Organic Carbon, and Dye Sorption between Flow and No-Flow Regions of Unsaturated Soil

L. A. Gaston* and M. A. Locke

### Abstract

**Transport models in which the liquid phase is partitioned between conducting and nonconducting regions allow the possibility that degradation and sorption are different in these regions. However, there is little information on biological or chemical differences between conducting and nonconducting regions of the soil matrix. Previous work by the authors on $Br^-$ transport through unsaturated, intact soil cores of Dundee silty clay loam (fine-silty, mixed, active, thermic Typic Endoaqualf) indicated non-equilibrium conditions that could be well-described by a two-region model. Fitted parameters indicated little solute transfer between flow regions, suggesting that dye movement in unsaturated soil might delineate conducting and nonconducting regions of this soil. Steady-state, unsaturated flow was established in intact cores (10 by 30 cm) of the Dundee soil, then $Br^-$ and erioglaucine dye were displaced through these cores. The soil cores were then sectioned into 5-cm segments and stained soil was separated from unstained soil. Microbial biomass C, organic C, and dye sorption $K_D$ ($= g_{sorbed}\ kg_{soil}^{-1}/g\ L^{-1}$) values for stained and unstained soil were determined. Stained soil had higher microbial biomass C but generally lower organic C and lower affinity for dye sorption than unstained soil from the same depth increment. Fraction of immobile water, dispersion, and mass transfer between conducting and nonconducting regions were consistent with previous results.**

THE two-region model of van Genuchten and Wierenga (1976) allows for different sorption $K_D$ values in conducting and nonconducting regions. An extension of this model (van Genuchten and Wagenet, 1989) includes different first-order degradation constants for these two regions. Where the mobile water region is clearly defined as interaggregate or biopore space and aggregate surface or biopore lining can be removed and analyzed separately from the bulk soil (Stehouwer et al., 1993, 1994), sorption or degradation in the water-conducting or -nonconducting portion of the matrix may be directly measured. Elsewhere, dye solutions applied to the soil surface may be used to identify preferential flow paths, then stained and unstained soil separated and analyzed. Using this technique, Bundt et al. (2001b) found that measures of biological activity and chemical properties associated with organic matter were higher in preferential flow paths of a forest soil than in the surrounding matrix. Similarly, concentrations of polyaromatic hydrocarbons, polychlorinated biphenyls (Bundt et al., 2001a), and radionuclides from atmospheric deposition (Bundt et al., 2000) were also higher in flow paths.

Gaston and Locke (1996) found that transport of a weakly sorbed pesticide through intact cores of water-unsaturated Dundee soil was affected by preferential flow but were unable to obtain meaningful (low standard error) estimates of degradation constants for conducting and nonconducting regions by curve fitting. Clearly, if sorption and degradation in these regions differ substantially but differences are ignored, chemical movement may not be accurately modeled. This study was undertaken, therefore, as a follow-up to Gaston and Locke (1996) and related studies (Gaston et al., 1996; Gaston and Locke, 2000) to measure biological or chemical differences (if any) between conducting and nonconducting regions of unsaturated Dundee soil that may influence chemical fate.

### Materials and Methods

#### Tracer and Dye Movement through Intact Columns

Duplicate intact soil cores (10 cm diam. by 30 cm long) in PVC were collected from conventional-tillage (CT) and no-tillage (NT) plots of a long-term research field on Dundee silty clay loam. Steady-state unsaturated flow (0.005 *M* $CaCl_2$) was established through the cores (see Gaston and Locke, 1996, for design). A 10-mL pulse of 1.00 *M* KBr was applied to the surface of each core and eluted. Effluent was collected in fractions and analyzed for $Br^-$ by ion chromatography. The two-region model of van Genuchten and Wagenet (1989), as adapted by Gaston and Locke (1996), was fit to $Br^-$ effluent data to estimate fraction of immobile water and dispersion and mass transfer coefficients.

Previous work (Gaston and Locke, 1996, 2000; Gaston et al., 1996) indicated little mass transfer between regions. Thus, dye patterns might visually distinguish conducting and nonconducting regions so that these could be physically separated. Following $Br^-$ elution (to prevent interaction; Allaire-Leung et al., 1999), about 2 L of erioglaucine (FD&C Blue No. 1; 2.4 g $L^{-1}$ in 0.005 *M* $CaCl_2$) were applied to soil cores (see Flury and Flühler, 1994, for various properties of this dye). After either dye application to cores CT 1 and NT 1 or after an additional 4 L of 0.005 *M* $CaCl_2$ to cores CT 2 and NT 2, flow was stopped and the apparatus dismantled. Cores were sectioned into 5-cm increments.

L.A. Gaston, Dep. of Agronomy, 104 Sturgis Hall, LSU AgCenter, Baton Rouge, LA 70803. M.A. Locke, USDA-ARS Southern Weed Science Unit, P.O. Box 350, Stoneville, MS 38776. Received 7 Aug. 2001. *Corresponding author (lgaston@agctr.lsu.edu).

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**Abbreviations:** CT, conventional tillage; NT, no tillage.

### Analyses of Stained and Unstained Soil

Stained soil was separated from unstained soil and the wet mass of each recorded. Moisture content was determined on subsamples and the remainder stored at 4°C. Subsamples of stained soil were repeatedly extracted with 80:20 methanol to 0.005 *M* $CaCl_2$ (10:1 solution to soil) to recover the dye. Extraction was considered complete when colorimetric analysis (Flury and Flühler, 1995) of extract gave zero absorbance. Extracts were combined and average concentration of erioglaucine measured to determine the total mass of dye per mass of stained soil.

Microbial biomass C was determined by the chloroform fumigation method (Voroney and Paul, 1984). To correct for possible effect of dye on microbial populations in the stained samples, an equal mass of dye was added to unstained samples and these were incubated for one week prior to fumigation. Biomass C was determined on duplicate samples of stained and unstained soil. Organic C in duplicate samples was determined by wet digestion (Nelson and Sommers, 1982). Measured organic C in stained samples was corrected for measured dye content. Dye sorption in stained samples was estimated from measured extractable mass of dye and solution concentration of dye desorbed by shaking duplicate samples with 0.005 *M* $CaCl_2$ (1:2 soil to solution) for 48 h. Duplicate unstained samples were shaken with dye solutions that contained a mass of dye equal to that in the stained soil from the same depth increment. Sorption $K_D$ values for initially unstained soil were calculated from change in solution concentration.

## Results and Discussion

### Bromide Elution

Intact cores of Dundee soil previously examined (Gaston and Locke, 1996, 2000; Gaston et al., 1996) exhibited preferential flow. Transport parameters estimated from $Br^-$ elution from the CT and NT columns used in this study were generally consistent with previous data (Table 1); however, the estimated fraction of immobile water was somewhat lower. Data for 18 intact soil cores (Table 1) suggest that tillage had negligible effect on non-equilibrium transport parameters.

**Table 1. Best-fit values for column-average fraction of immobile water ($f = \theta_{IMj}/\theta_j$), dispersion ($\psi = D_j\theta_{Mj}/q$), and mass transfer between mobile and immobile water regions ($\varphi = \alpha_j\theta_{Mj}/q$) based on $Br^-$ elution from vertically heterogenous soil cores, where *j* refers to any vertical increment, $\theta_j$ is a total volumetric water content, $\theta_{Mj}$ is mobile region volumetric water content, $D_j$ (cm$^2$ d$^{-1}$) is dispersion coefficient, $q$ (cm d$^{-1}$) is water flux, and $\alpha_j$ (d$^{-1}$) is mass transfer coefficient (Gaston and Locke, 1996). Previous data (Gaston and Locke, 1996, 2000; Gaston et al., 1996) are average values for conventional-till (CT) and no-till (NT) soil cores.**

| Study | Soil core(s) | Fraction of immobile water, $f$ | Dispersion, $\psi$ | Mass transfer, $\varphi$ |
|---|---|---|---|---|
| | | | cm | cm$^{-1}$ |
| Current | CT 1 | 0.08 ± 0.01† | 2.8 ± 0.4 | 0.001 ± 0.001 |
| | CT 2 | 0.08 ± 0.01 | 1.2 ± 0.1 | 0.001 ± 0.001 |
| | NT 1 | 0.11 ± 0.01 | 1.4 ± 0.1 | 0.001 ± 0.001 |
| | NT 2 | 0.05 ± 0.01 | 1.5 ± 0.2 | 0.004 ± 0.002 |
| Previous | CT 3–9 | 0.18 ± 0.01 | 1.2 ± 0.1 | 0.005 ± 0.002 |
| | NT 3–9 | 0.17 ± 0.03 | 3.0 ± 0.7 | 0.015 ± 0.009 |

† Standard error, current study, and average standard error, previous studies.

### Characterization of Stained and Unstained Samples

Erioglaucine movement in the soil cores was slow, consistent with earlier transport studies with this dye (Andreini and Steenhuis, 1990; Flury and Flühler, 1995; Allaire-Leung et al., 1999). No staining was observed below the 20-cm depth despite application of more than 6 pore volumes to cores CT 2 and NT 2. Staining in several core sections was largely associated with fine root channels or narrow earthworm borrows; however, staining generally was not associated with biopores. All soil above the 5-cm depth in cores CT 1 and NT 1 was deeply stained, however, there were areas in the 0- to 5-cm depth of cores CT 2 and NT 2 from which dye had been partially eluted. But even in these, staining was too pervasive to allow separation of stained from unstained soil in the 0- to 5-cm depth. Therefore, only soil between 5 and 20 cm was used to measure differences in microbial biomass C, organic C, and $K_D$ values.

Differences in microbial biomass C between stained and unstained soil (Table 2) indicated significantly greater biomass C in stained soil ($p < 0.01$). This result is consistent with expected better aeration and supply of dissolved organic substrates in the water-conducting region. Bundt et al. (2001b) obtained similar results for a forest soil.

Differences in organic C between stained and unstained portions of each core section (Table 2) indicated more organic C in the unstained soil ($p < 0.10$). This result may reflect lower microbial activity in the unstained regions of soil (as suggested by lower microbial biomass) or indicate that organic matter in nonconducting regions is relatively protected from decomposition by soil microorganisms. Gregorich et al. (1989) and Beare et al. (1994), for example, have shown that disruption of soil aggregates accelerates decomposition of organic matter otherwise protected from microbial attack.

The difference, Δ organic $C_{unstained-stained}$, was greater for the NT cores (average for NT, 1.16, and average for CT, 0.05 mg kg$^{-1}$; $p < 0.05$). Little difference in organic C between conducting and nonconducting regions of the CT soil probably reflected continual disturbance of the upper soil by tillage. Higher organic C in the NT matrix soil, compared with flow paths, is opposite to the finding of Bundt et al. (2001b). However, unlike Bundt et al. (2001b), this study constrained flow to unsaturated conditions. Whereas higher organic C may occur in macroporous zones than in matrix soil due to

**Table 2. Comparisons of chemical and biological data for stained and unstained soil.**

| Core† | Depth | ΔBiomass C, stained − unstained | ΔOrganic C, unstained − stained | $K_D$ ratio, unstained/stained |
|---|---|---|---|---|
| | cm | mg C kg$^{-1}$ soil | g C kg$^{-1}$ soil | |
| CT 1 | 5–10 | 250 | 0.5 | 1.2 |
| | 10–15 | 100 | −0.1 | 4.3 |
| CT 2 | 5–10 | 140 | −0.2 | 5.2 |
| | 10–15 | 130 | 0.0 | 5.5 |
| NT 1 | 5–10 | 250 | 1.0 | 1.4 |
| | 10–15 | 200 | 1.6 | 1.9 |
| | 15–20 | 140 | 1.9 | 1.8 |
| NT 2 | 5–10 | 200 | 1.6 | 2.6 |
| | 10–15 | 170 | −0.3 | 6.3 |

† CT, conventional till; NT, no till.

deposition by roots and transport of organic matter from the soil surface (Bundt et al., 2001b), lower organic C apparently occurred along unsaturated preferential flow paths than in surrounding soil.

Based on results of Flury and Flühler (1995), higher $K_D$ values in unstained than in stained soil were expected due to the higher organic C content of the unstained soil. Relative $K_D$ [$K_{D(unstained)}/K_{D(stained)}$] values in core sections (Table 2) were significantly greater than 1 ($p < 0.01$). Given the exhaustive extraction of the stained soil sample to recover erioglaucine dye, it seems unlikely that mass of dye in the stained soil and, therefore, dye sorption in the stained soil, was underestimated. Furthermore, the total mass of dye recovered from each core accounted for the total mass applied. However, greatest $K_{D(unstained)}$ to $K_{D(stained)}$ ratios were found for core sections with greatest relative organic C in the stained soil (Table 2). To the extent values for organic C in these samples reflected failure to account for all sorbed dye, inaccurately low estimates of $K_D$ values for the stained soil and inflated $K_D$ ratios would have resulted.

These results indicate that microbial biomass C, organic C content (for NT soil), and dye sorption are different in water-conducting and nonconducting regions of unsaturated Dundee soil. However, these results may be conservative estimates of differences that occur in the field. The lateral size (10 cm in diameter) of soil cores may have partially truncated natural unsaturated preferential flow paths downward, intercepted others that originated outside the core volume, and forced flow along paths connecting these discontinuities that would not have occurred in the field. On the other hand, only soil down to 20 cm was stained so that depth of identifiable flow path compared with core diameter was not very long. Accordingly, flow along such nonnatural paths may have been minimal.

Lower organic C in the conducting than in the nonconducting region implies greater potential chemical mobility than would be predicted if sorption was assumed equal in both regions. However, larger microbial biomass in the conducting region suggests potentially faster degradation. The extent to which these opposing effects offset one another probably depends on particular solute–soil interactions and substrate-specific biodegradation rate.

## REFERENCES


Allaire-Leung, S.E., S.C. Gupta, and J.F. Moncrief. 1999. Dye adsorption in a loam soil as influenced by potassium bromide. J. Environ. Qual. 28:1831–1837.

Andreini, M.S., and T.S. Steenhuis. 1990. Preferential paths of flow under conventional and conservation tillage. Geoderma 46:85–102.

Beare, M.H., M.L. Cabrera, P.F. Hendrix, and D.C. Coleman. 1994. Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils. Soil Sci. Soc. Am. J. 58:787–795.

Bundt, M., A. Albrecht, P. Froidevaux, P. Blaser, and H. Flühler. 2000. Impact of radionuclide distribution in soil. Environ. Sci. Technol. 34:3895–3899.

Bundt, M., M. Krauss, P. Blaser, and W. Wilcke. 2001a. Forest fertilization with wood ash: Effect on the distribution and storage of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). J. Environ. Qual. 30:1296–1304.

Bundt, M., F. Widmer, M. Pesaro, J. Zeyer, and P. Blaser. 2001b. Preferential flow paths: Biological 'hot spots' in soil. Soil Biol. Biochem. 33:729–738.

Flury, M., and H. Flühler. 1994. Brilliant blue FCF as a dye tracer for solute transport studies—A toxicological review. J. Environ. Qual. 23:1108–1112.

Flury, M., and H. Flühler. 1995. Tracer characteristics of brilliant blue FCF. Soil Sci. Soc. Am. J. 59:22–27.

Gaston, L.A., and M.A. Locke. 1996. Bentazon mobility through intact, unsaturated columns of conventional- and no-till Dundee soil. J. Environ. Qual. 25:1350–1356.

Gaston, L.A., and M.A. Locke. 2000. Acifluorfen sorption, degradation and mobility in a Mississippi Delta soil. Soil Sci. Soc. Am. J. 64:112–121.

Gaston, L.A., K.N. Reddy, and M.A. Locke. 1996. Norflurazon fate in a Mississippi Delta soil. p. 29. *In* 1996 Agronomy abstracts. ASA, Madison, WI.

Gregorich, E.G., R.G. Kachanoski, and R.P. Voroney. 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. J. Soil Sci. 40:649–659.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 539–579. *In* A.L. Page (ed.) Methods of soil analysis. Part 2. Chemical and microbiological methods. 2nd ed. Agron. Monogr. 9. ASA, Madison, WI.

Stehouwer, R.C., W.A. Dick, and S.J. Traina. 1993. Characteristics of earthworm burrow lining affecting atrazine sorption. J. Environ. Qual. 22:181–185.

Stehouwer, R.C., W.A. Dick, and S.J. Traina. 1994. Sorption and retention of herbicides in vertically oriented earthworm and artificial burrows. J. Environ. Qual. 23:286–292.

Van Genuchten, M.Th., and R.J. Wagenet. 1989. Two-site/two-region models for pesticide transport and degradation: Theoretical development and analytical solutions. Soil Sci. Soc. Am. J. 53:1303–1310.

Van Genuchten, M.Th., and P.J. Wierenga. 1976. Mass transfer studies in sorbing porous media: 1. Analytical solutions. Soil Sci. Soc. Am. J. 40:473–480.

Voroney, R.P., and E.A. Paul. 1984. Determination of $k_C$ and $k_N$ in situ for calibration of the chloroform-fumigation incubation method. Soil Biol. Biochem. 16:9–14.