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The Role of Microbes Associated with Organic and Inorganic Substrates
in Phosphorus Spiralling in a Woodland Stream.¹

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¹Research supported by the National Science Foundation's Ecosystem Studies Program under Interagency Agreement No. DEB-78-03012 with the U.S. Department of Energy under contract W-7405-eng-26 with Union Carbide Corporation. Publication No. 1579, Environmental Sciences Division, Oak Ridge National Laboratory.

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Introduction

Because of the continuous, unidirectional flow of water and particulate material in streams, the "cycling" of nutrients through biotic compartments (algae, bacteria, fungi, and consumers) on the stream bottom is a spatially-dependent process and can be described as an imaginary spiral (Newbold et al. In Press). A nutrient atom in solution that has entered a stream in groundwater, surface runoff, or through mineralization of, and desorption from, sediments will travel some distance downstream before it is sorbed by particulate materials and associated microbes (algae, bacteria, fungi) on the stream bottom. We refer to this expected travel distance of a nutrient in solution before being sorbed by stream sediments as the uptake length. It can be estimated from measurable fluxes as the ratio of the downstream flux of dissolved nutrient in water (g s^{-1}) to the uptake flux from water to particulates per unit length of stream bottom ($\text{g s}^{-1}\text{m}^{-1}$).

Once sorbed by particulate materials and associated microbes on the stream bottom, the nutrient can be released directly back to water (through metabolic turnover, desorption, and mineralization), ingested and assimilated by consumers (e.g., grazers, shredders), or remain in the particulate phase. While in the particulate or consumer phase, the nutrient is also subject to downstream transport in the suspended and bed load (e.g., saltation, resuspension, and sloughing) and through drift of consumers. The expected distance a nutrient travels in the particulate and consumer phase before returning to water is referred to as the turnover length. We can estimate this as the ratio of the downstream flux of the exchangeable fraction of nutrient associated with the particulate and consumer phase (g s^{-1}) to the release flux of the nutrient per unit length of stream bottom from particulates and consumers back to water ($\text{g s}^{-1}\text{m}^{-1}$). The sum of the uptake length and

the turnover length is the distance required to complete the spatially-scaled cycle in a stream or river and is referred to as the spiralling length. The shorter the spiralling length, the more times a nutrient can be utilized within a given reach of stream. If spiralling is primarily a biological process, involving absorption and turnover of nutrients by the microbial community on organic and inorganic substrates on the stream bottom, then spiralling length is an index of nutrient utilization relative to the total downstream flux of a nutrient. Further, if productivity of the microbial community is a stoichiometric function of the downstream flux of the nutrient, then spiralling length is also an index of productivity.

Using $^{32}\text{PO}_4$, we estimated the spiralling length of $\text{PO}_4\text{-P}$ in Walker Branch, a small, first-order woodland stream in east Tennessee, to be 176 m in July (Newbold et al. In Press). Detrital decomposition rates and algal biomass in this stream are phosphorus limited (Elwood et al., In Press). The uptake length of phosphorus was estimated to be 164 m, while the turnover length was only 12 m. Coarse and fine particulate organic matter (CPOM and FPOM, respectively) and aufwuchs associated with inorganic substrates accounted for over 90% of the measured uptake of $^{32}\text{PO}_4$ from stream water. The relative importance of biological uptake versus abiotic sorption onto stream sediments however, was not defined.

In this paper, we present results of laboratory and field experiments to determine if nutrient spiralling is primarily a biological process. The experiments were conducted to examine the role of microbial uptake and abiotic sorption onto organic and inorganic substrates in the uptake of $\text{PO}_4\text{-P}$ in Walker Branch, to estimate the total, microbial, and adsorptive pool sizes of exchangeable phosphorus associated with FPOM from this stream,

and to measure the turnover rate of $\text{PO}_4\text{-P}$ by live and sterile inorganic substrates in Walker Branch.

Method and Materials

Microbial uptake and abiotic sorption of $\text{PO}_4\text{-P}$ by organic and inorganic substrates from Walker Branch were determined by measuring the loss of $^{32}\text{PO}_4$ from water in stirred microcosms containing either non-sterile (i.e., live) or sterile substrates. Fine particulate organic matter (FPOM) consisting of fragmented leaf detritus was collected from Walker Branch and wet sieved through a 1-mm mesh screen. Sufficient FPOM was added to four microcosms, each containing 1250 ml of filtered stream water, to attain a solution to solid (dry weight) ratio of approximately 2000:1. Three of the microcosms were maintained at 4°C and three at 16°C in environmental chambers with no lights. One microcosm containing FPOM at each temperature was sterilized with 0.5% formalin. A control microcosm containing only filtered, unsterilized stream water was also run at each of the two temperatures.

One day after adding the FPOM to the microcosms, 1 microcurie (μCi) of ^{32}P (as carrier-free $\text{K}_2^{32}\text{PO}_4$) was added to each treatment and the control. Water samples were collected and filtered at approximately 10-minute intervals during the first hour, 1 to 3 hour intervals during the next 12 hours, and 1 to 2 day intervals thereafter. Samples were membrane filtered (Nuclepore, 0.4 μ pore size) immediately upon collection and analyzed for soluble reactive phosphorus (SRP) using the ascorbic acid reduction method (EPA 1974). ^{32}P was counted in Insta-gel (Packard Instrument Co., Downers Grove, Illinois) using a liquid scintillation spectrometer. The specific activity of ^{32}P ($^{32}\text{P}/\text{total P}$) in both water and FPOM in all microcosms was determined when the ^{32}P concentration

in water had reached steady-state. Total P associated with FPOM was measured using the ignition method of Anderson (1976).

Abiotic sorption and biotic uptake onto inorganic substrates in Walker Branch were estimated using the same procedure as that for FPOM. The loss of $^{32}\text{PO}_4$ from filtered stream water containing either non-sterile or sterile rocks (chert, the predominant inorganic substrate in Walker Branch) was followed until a steady-state concentration of the isotope in all treatments was attained. Rocks approximately 10 cm in diameter were collected from the stream and transferred to microcosms in environmental chambers, maintained at 10°C with an 8-hour photoperiod. Each microcosm contained 1250 ml of filtered stream water and two rocks. One day after the rocks were introduced into the microcosms, approximately 1.7 μCi of carrier-free $^{32}\text{PO}_4$ was added to the non-sterile and sterile treatments and to a non-sterile control containing only filtered stream water. Water samples were collected, filtered, and assayed as before for ^{32}P and SRP.

After steady-state had been reached, the non-sterile and sterile rocks were gently rinsed with filtered stream water. The relative activity of ^{32}P (cpm) on each rock was measured using a Geiger-Muller (G-M) counter. The rocks were then transferred to filtered stream water and returned immediately to Walker Branch. Turnover of phosphorus associated with each rock was measured by following the decline in the relative activity of ^{32}P on each rock. In order to reduce microbial growth and activity on the sterile substrates once they were returned to the stream, the sterile rocks were immersed in 0.5% formalin for approximately 5 minutes on the days they were counted.

Results and Discussion

As Figure 1 shows, the loss of $^{32}\text{PO}_4$ from water containing non-sterile FPOM was substantially greater than the loss in the sterile and control systems at both 4° and 10°C. In the sterile treatments the loss of ^{32}P presumably was a result of abiotic sorption onto the FPOM. While there clearly was some adsorption of phosphorus, the difference in ^{32}P concentration between the non-sterile and sterile FPOM at equilibrium shows that uptake of the tracer from water was due predominantly to absorption by bacteria, fungi, and other microbes associated with the FPOM. The more rapid uptake of ^{32}P by live FPOM at 10°C, compared to that at 4°C, is consistent with the known temperature dependence of microbial uptake (e.g., Chen, 1974), providing additional evidence that the predominant mechanism of phosphorus uptake in the non-sterile treatments was absorption. Differences in the steady-state concentrations of ^{32}P in water among treatments at the two temperatures are probably due to differences in the solution to solid ratio in the microcosms.

Of the total phosphorus associated with non-sterile FPOM, approximately 34% of it is estimated to be exchangeable with $\text{PO}_4\text{-P}$ in water (Table 1). The difference in exchangeable phosphorus pools between non-sterile and sterile FPOM can be attributed to absorption of phosphorus by microbes growing on the surface of these particles. Thus, approximately 31% of the total P associated with FPOM is a result of microbial uptake, while only 3% is a result of adsorption and other abiotic exchange processes. Expressed in terms of the exchangeable phosphorus pool, 91% of the total exchangeable pool is a result of microbial uptake, while the remaining 9% is attributed to abiotic exchange mechanisms. The fraction of phosphorus associated with FPOM which did not exchange with PO_4 in water either has such a slow turnover rate that its exchange would

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not be measured within the time frame of this experiment, or it is fixed irreversibly in the particle matrix. In either case, these results suggest that a substantial fraction of the P associated with decomposing organic matter in Walker Branch is not being cycled in the stream and, therefore, does not contribute to productivity of the ecosystem.

Differences in the loss of $^{32}\text{PO}_4$ from water in microcosms containing non-sterile and sterile rocks (Figure 2) indicate that absorption of phosphorus by the aufwuchs community is also the predominant mechanism for uptake onto inorganic sediments. Concentrations of ^{32}P in water in the sterile treatment after 9 days indicate little or no net sorption of ^{32}P onto the rocks. However, this is an artifact caused by evaporation of water from the microcosms.

Our findings of the relative importance of biological and abiotic processes in phosphorus uptake are consistent with Gregory's (1978) conclusion that physical sorption was generally less than 20% of phosphorus uptake onto stream sediments. Meyer (1979), however, concluded that microbial uptake played a relatively minor role in phosphorus sorption by inorganic sediments in Bear Brook, New Hampshire. Her results are based on measurements of the net uptake over 24 hours of $\text{PO}_4\text{-P}$ from water containing non-sterile and sterile sediments. The concentrations of $\text{PO}_4\text{-P}$ to which the sediments were exposed, however, were significantly greater than the ambient levels in the stream from which the sediments were collected. If the experimental concentrations of $\text{PO}_4\text{-P}$ in water substantially exceeded the half-saturation constant for microbes growing on these sediments, then the intracellular turnover time of P would probably be short (Law, et al. 1976). Therefore, little or no difference in net uptake of phosphorus between non-sterile and sterile sediments would be observed.

Loss of ^{32}P from the non-sterile and sterile chert returned to Walker Branch occurred at rates which can be described by the exponential decay function, $Y = A_0 e^{-kt}$, where Y is the amount of ^{32}P (cpm) associated with a rock at time t (days) after the rock was returned to the stream, A_0 is the initial ^{32}P activity on the rock (i.e., when removed from the microcosm), and k is the turnover rate of phosphorus on the rock (day^{-1}). The loss of ^{32}P associated with the two non-sterile rocks occurred at rates that were significantly faster (0.123 day^{-1} and 0.140 day^{-1}) than for the two sterile rocks (0.025 day^{-1} , 0.058 day^{-1}) (Figure 3), suggesting that the turnover of phosphorus on inorganic substrates also is increased by biological processes. In the absence of microbial activity, the only mechanism of phosphorus turnover by the sterile rocks is desorption. Regressions of ^{32}P retention by the sterile rocks (Figure 3) show that the turnover rate of phosphorus associated with each rock was constant throughout the 18-day period of measurement. This constancy, combined with the fact that these rocks were sterilized at different intervals which allowed different times for microbial growth, suggests that desorption of phosphorus from the sterile rocks was not influenced by microbial activity.

The calculated turnover rates of phosphorus on non-sterile chert (Figure 3) are comparable to earlier estimates of the turnover rates of phosphorus associated with aufwuchs in summer and early fall (Elwood and Nelson 1972). The latter estimates are based on losses of ^{32}P from aufwuchs removed from sandstone following releases of $^{32}\text{PO}_4$ directly to the stream. The comparability of these estimated turnover rates indicates that the loss of ^{32}P from the non-sterile rocks was a result of turnover of phosphorus associated with the aufwuchs and not desorption from the rock surface.

The faster loss of ^{32}P from the non-sterile rocks may have been caused by turnover of the growing microbial biomass (through grazing and sloughing), turnover of intra-cellular phosphorus, or both. The standing crop of aufwuchs on inorganic substrates in Walker Branch is low, averaging 200 mg/m^2 (Elwood and Nelson 1972). At these low biomass levels, sloughing of aufwuchs probably is not a significant mechanism of loss. Estimates of grazing on aufwuchs in this stream, however, indicate that at certain times grazing rates may exceed aufwuchs production rates (Elwood and Nelson 1972). The faster turnover of phosphorus on the non-sterile rocks, which presumably had more biomass than the sterile chert, thus may have been caused by grazing of aufwuchs on these non-sterile substrates. Grazers also may indirectly enhance the turnover of phosphorus in microbial communities by stimulating the cycling of P in bacteria and algae. Barsdate et al. (1974) and Fenchel and Harrison (1975), for example, demonstrated that phosphorus turnover was more rapid in systems containing protozoan grazers than in systems without grazers. Whatever the cause of the differences we observed in phosphorus turnover rates between non-sterile and sterile chert, the faster uptake and turnover on non-sterile substrates indicates that both processes are controlled predominantly by biological mechanisms.

The similarity in loss of $^{32}\text{PO}_4$ from water within the first 30 minutes between the sterile and non-sterile FPOM (Figure 1) and between the sterile and non-sterile chert (Figure 2) indicates that the mechanism responsible for the initial uptake of phosphorus was the same in the sterile and the non-sterile systems. Since there was no absorption in the sterile treatments, this suggests that the initial uptake of $^{32}\text{PO}_4$ in the non-sterile systems was adsorption onto living microbes followed by transport of the tracer into the cells. This mechanism of accumulation of phosphorus by

microbes, involving both passive adsorption to the cell surface followed by active transport into the cell, is consistent with observed uptake kinetics of phosphorus by bacteria (e.g., Chen 1974), where the maximum uptake velocity of phosphorus decreases as the number of uptake sites per cell is reduced. Thus, in the sterile systems, uptake sites on microbial cells either reach equilibrium with PO_4 in solution or become saturated because there is no active transport of phosphorus into the cells. In the non-sterile systems, however, uptake continues as phosphorus adsorbed on the cell surface is transported into the cell. This passive-active mechanism of phosphorus uptake implies that in the presence of living microbes, adsorption onto living cells followed by desorption directly back to water is probably not an important mechanism of phosphorus spiralling in streams.

Acknowledgements

We thank Carol Hom for her help in collecting and processing samples and P. J. Mulholland, R. R. Turner, and W. Van Winkle for reviewing the manuscript.

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Table 1. Pool sizes of exchangeable phosphorus associated with fine particulate organic matter (FPOM) from Walker Branch. Estimates are based on differences in specific activities of ^{32}P between water and non-sterile and sterile FPOM at isotopic equilibrium;

	Exchangeable P (as % of total P associated with FPOM)
Total (biotic + abiotic)	33.6
Biotic	30.7
Abiotic	2.9

Figure Legends

- Figure 1. Concentrations of ^{32}P in filtered stream water in microcosms containing non-sterile (L) and sterile (S) fine particulate organic matter from Walker Branch or filtered, non-sterile stream water only (C) as a function of time after the microcosms were spiked with $^{32}\text{PO}_4$.
- Figure 2. Concentrations of ^{32}P in water in microcosms containing non-sterile and sterile rocks (chert) from Walker Branch as a function of time after the microcosms were spiked with $^{32}\text{PO}_4$.
- Figure 3. Relative activity of ^{32}P retained on chert rocks as a function of time after the rocks were removed from microcosms and returned to Walker Branch. Turnover rates of phosphorus based on the slope of the regressions of ^{32}P retained versus time in the stream are -0.123 day^{-1} ($r^2 = .972$) and -0.140 day^{-1} ($r^2 = 0.989$) for the non-sterile chert and -0.025 day^{-1} ($r^2 = 0.922$) and -0.058 day^{-1} ($r^2 = 0.856$) for the sterile chert.

Figure 1

Figure 2

Figure 2