Microbial Processes in the Vadose Zone

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ABSTRACT

Surface soils and their microbiology have been studied for decades. However, subsurface soil, more broadly referred to as the vadose zone, is of increasing interest to microbiologists. The vadose zone, extending from the terrestrial surface to the groundwater table, is rich in microbes of many types. This review summarizes what is known about the abundance and diversity of microbes in the vadose zone and the environmental factors that influence vadose zone microbes and microbial processes. We discuss the roles of vadose zone microbes in nutrient cycling as well as their importance in pollutant remediation. We address a number of fundamental questions in vadose zone microbial ecology, including: What do we need to learn about vadose zone microbes to improve our ability to predict the fates of pollutants? How different are microbial communities and microbial activities in the terrestrial subsurface compared with surface soil? Numerous questions and arguments justify "deepening" soil microbiology's spatial context to include the whole unsaturated subsurface.

Nutrient cycling catalyzed by microbes is the foundation for sustained life on our planet. In agriculture, knowledge of stoichiometry and kinetics associated with microbially catalyzed nutrient cycling can be applied to refine fertilization practices (Matson et al., 1998). However, nutrients of all kinds escape to soil depths far below plant rooting zones. For example, considerable attention has been focused on problems associated with the leaching of nitrate and herbicides into groundwater (as summarized by Albrechtsen et al., 2001). A wide range of chemical pollutants are either released from buried tanks or originate in surface spills that migrate to deeper horizons. Yet the field of soil microbiology has traditionally focused on the plant root zone and has largely ignored the entirety of the vadose zone, the unsaturated zone extending from the soil surface to the groundwater table. Given the vastness of the vadose zone, the growing recognition that it harbors diverse communities of microbes, and our reliance on the vadose zone as a natural "reactor" for pollutant attenuation, we need to "deepen" the spatial context of soil microbiology to better understand the microbial ecology of the unsaturated subsurface.

To date, much of the research in vadose zone microbiology has focused on documenting the presence, abundance, diversity, and viability of vadose zone microbes. This emphasis on largely descriptive research is striking given that vadose zone microbial processes, such as biodegradation, hold great societal importance. What do we need to know about vadose zone microbes to enhance their ability to degrade pollutants such as pesticides or spilled petroleum? This review focuses on microbial processes in the vadose zone, including the environmental controls, the quantitative modeling of vadose zone microbial processes, and the information required to improve existing models.

Excellent reviews of vadose zone microbiology (e.g., Ghiorse and Wilson, 1988; Kieft and Brockman, 2001; Madsen, 1995) have preceded this review. Some prior reviews (e.g., Madsen, 2000) do not draw a crisp distinction between the microbiology of the unsaturated vs. saturated terrestrial subsurface. Hydrologically, the vadose zone is connected to the saturated zone. However, except for the fluctuating capillary interface, the vadose zone is distinct in that the microbes residing there live under conditions of relatively low water availability. For this reason, we exclude discussion of groundwater microbial processes in this review. Also, this review does not attempt to summarize the vast literature of soil microbiology. For an introduction to the soil microbiology literature and exposure to key principles, the reader is referred to either Paul and Clark's (1989) textbook or to many other excellent texts on the subject (e.g., Richards, 1987; Tate, 1995).

ENVIRONMENTAL FACTORS AFFECTING VADOSE ZONE MICROBIAL PROCESSES

The physical and chemical characteristics of deep soil environments are very different from the surface. Because microbes are highly responsive to their environments, examining the environmental factors that are characteristic of the vadose zone provides a context for understanding the distribution and functioning of vadose zone microbes, the main thrust of this review. The factors affecting microbial physiology in the vadose zone are similar to those affecting microbes everywhere: the availability of water, C, energy, terminal electron acceptors, and other nutrients and other environmental factors such as pH and temperature. Compared with surface soils, the magnitude and variations of these factors are different in deeper soils. For example, both moisture and temperature are highly variable in surface soils, but the variability is generally reduced with vadose zone depth (e.g., Fierer, 2003; Hendry et al., 1999; Hillel, 1980; Jury et al., 1991). Compared with surface soils, deep (Kieft et al., 1993) and near surface (Fierer et al., 2003a; Kieft et al., 1993) vadose zone materials harbor

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Published in Vadose Zone Journal 4:1–21 (2005).

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Abbreviations: CFU, colony forming unit; DGFA, diglyceride fatty acids; DOC, dissolved organic C; EPS, exopolymeric substances; MPN, most probable number; OM, organic matter; OTU, operational taxonomic unit; PCE, perchloroethylene; PLFA, phospholipid fatty acids; SIR, substrate induced respiration; TCE, trichloroethylene; TRFLP, terminal restriction fragment length polymorphisms; VC, vinyl chloride.

microbes that are desiccation tolerant, indicating that they are frequently exposed to low water content and low water potentials. Also, vadose zone microbes, compared with microbes in surface soils, are comparatively more sensitive to shifts in temperature and nutrient availability (Fierer et al., 2003a), which can be explained by the relatively more stable conditions of deeper soils.

Most solution-phase resources in surface soil (dissolved C, N, and other nutrients) are plant derived and are readily biodegraded. Thus, these resources are abundant in surface soil, but their concentrations decline steeply with depth to low, equilibrium values in deep soils (e.g., Fierer et al., 2003b). Total soil C (Bennema, 1974; Spycher et al., 1983), organic C (Arrouays and Pelissier, 1994; Bennema, 1974; Richter and Markewitz, 1995; Trumbore et al., 1995), and mineralizable C (Ajwa et al., 1998) decrease dramatically with depth. Organic C in deep soils also appears to be very old, with a radiocarbon age of several thousand years (Rumpel et al., 2002). The pattern of C declination with depth has been modeled reasonably well as a power function (Bennema, 1974):

$$C_y = \alpha y^{\beta} \quad [1]$$

where C_y is C concentration at depth y, α is a constant for a profile and for specific units of C_y and y, β (negative in sign) is a constant for a profile and for specific units of C_y and y, and y is depth.

Alternatively, the organic C depth dependency may fit a more complex exponential function (Arrouays and Pelissier, 1994; Bernoux et al., 1998). An advantage of the exponential-type model is that, unlike the power model, it behaves normally at the surface ($y = 0$) (Bernoux et al., 1998). However, for an extensive dataset representing nearly 700 soil profiles, the power model was a better fit to the data and more accurate at predicting C concentrations (Jobbagy and Jackson, 2000). Power models are mainly applicable to the top 3 m (Bennema, 1974; Jobbagy and Jackson, 2000), where the sharpest C descent occurs.

Like C, total N (Spycher et al., 1983) and mineralizable N (Ajwa et al., 1998) also decline dramatically with depth. For thousands of soil profiles, P and K, in addition to C and N, showed a pattern of rapid declination with depth (Jobbagy and Jackson, 2001). As observed by Jobbagy and Jackson (2001), the nutrients that declined most dramatically with depth were those that were important to both plants and microbes. Magnesium and Ca appeared relatively unchanged with depth, whereas SO_4^-, Na, and Cl^- increased with depth down to 1 m (Jobbagy and Jackson, 2001).

Soil chemistry affecting substrate availability also changes dramatically with depth. Clay content, which controls availability of exchangeable ions, and organic matter (OM), which controls the abundance and availability of dissolved and available sorbed organic nutrients, both decrease with depth (Konopka and Turco, 1991). Soils with higher clay content appear to enhance starvation survival (England et al., 1993; Kieft et al., 1997), which may be due to the association of soil organic C with clay (Jobbagy and Jackson, 2000). The fact that clay, and thus also C, declines with depth may in part explain lower microbial abundances with depth in the vadose zone. As discussed in the next sections, the main influences of vadose zone material texture on microbial abundance appear to be due to textural relationships to soil C: low porosity rock, such as basalt, and higher porosity sands and silts with low C harbor fewer microbes as compared with buried soils ("highly developed" paleosols) that contain relatively more organic C (Brockman et al., 1992; Fredrickson et al., 1993; Kieft et al., 1998; Palumbo et al., 1994).

Compounding the effective low abundance of C at depth is that water availability limits C availability (Kieft et al., 1993). In fact, adding water can increase C availability, in turn increasing culturable counts of subsurface microbes by up to 200 times (Hickman and Novak, 1989). This fact is further supported by higher microbial biodegradation activity in the capillary fringe where the water table, rising and falling regularly, redistributes nutrients and possibly microbes (Lahvis et al., 1999; Widrig and Manning, 1995).

In addition to transporting C, water also transports microbes below the surface such that high recharge sites harbor different culturable bacteria as compared with low recharge sites (Brockman et al., 1992). Insight into whether recharge is actively occurring, and is thus a mechanism for microbial transport, can be gained by dating porewater and surrounding sediments. If the porewater is younger than the sediment, then modern recharge, a vehicle for microbial transport, is occurring (Kieft et al., 1998). Microbes can also be buried at depth during the formation of soils (Kieft et al., 1998), and thus recharge does not fully explain microbial distributions in all subsurface environments. Additionally, in arid vadose zones, water-mediated transport is limited by the filtration effects of porous media (Balkwill et al., 1998).

Soil gas changes with depth, but the changes are soil dependent. For example, CO_2 is 0.033% at the surface but, depending on the soil, ranges between 1 and 2.5% at depths spanning 2 to 20 m and deeper below the soil surface (Wood and Petraitis, 1984). Oxygen concentrations are atmospheric at the surface, but, depending on the soil, can decline to between 19 and 20% at 5 m below the surface, and to between 16 and 19% at 20 m below the surface (Wood and Petraitis, 1984). Alkalinity increases with depth in the vadose zone due to high CO_2 production and limited diffusional transport (Richter and Markewitz, 1995). In general, seasonal shifts in vadose zone respiration, as measured by CO_2 concentrations, follow seasonal shifts in mean daily temperature but not necessarily moisture content, which is more constant across seasons (Hirsch et al., 2002).

The vadose zone is assumed to be well aerated, and O_2 concentrations are nearly atmospheric in sandy soils at several meters depth (Hirsch et al., 2002). However, microbial processes tied to low O_2, such as anaerobic biodegradation of arachlor (Pothuluri et al., 1990) or microaerobic biodegradation of toluene (Holden et al., 2001), occur in vadose zone materials, which suggests that depending on soil texture and water content, low O_2 regions can exist in deeper soils just as they do in surface soils.

VADOSE ZONE MICROBIAL ABUNDANCE, DISTRIBUTION, AND COMMUNITY COMPOSITION

The vadose zone can be shallow (a few meters) to very deep (hundreds of meters; Stephens, 1996). The high densities of microbial biomass in surface horizons and the integrated biomass throughout the entire vadose zone contribute to microbial processes in the vadose zone. Before we focus on microbial processes within the vadose zone, we must first examine the abundance, distribution, and composition of microbial communities residing in the vadose zone.

Abundance and Distribution of Vadose Zone Microbes, from the Macro- to Microscales

Knowledge of the abundance and distribution of microbes in the vadose zone helps us ascertain the potential for microbially catalyzed reactions. Microbial abundance is generally measured in the laboratory using field cores that have been drilled horizontally through the face of a trench or vertically from the surface. Quantification of population units is either culture dependent, that is, by most probable number (MPN) and colony forming unit (CFU) enumeration, or culture independent, that is, by the direct counting of stained bacteria and nucleic acid probing. Another culture-independent method could be quantitative polymerase chain reaction (QPCR, also referred to as real time PCR or RT-PCR) of phylogenetically or functionally relevant genes, but at the time of this review the application of this method to soil appears to be limited to surface soils including the rhizosphere (e.g., Lee et al., 1996). Total biomass can be estimated by several methods, including the quantification of extractable phospholipid fatty acids (PLFAs) or diglyceride fatty acids (DGFAs), the assessment of total C liberated by chloroform fumigation, and the quantification of substrate uptake or respiration rates (substrate induced respiration, or SIR) during short incubation periods. The latter is essentially culture dependent and the former two are culture-independent measures of biomass. Each of the methods reported in Table 1 yields a different estimate of microbial abundance, and each has a distinct set of advantages and disadvantages. Not surprisingly, the abundance of vadose zone microbes has been measured and reported in almost as many ways as there are publications on the subject.

Microbial biomass is generally highest in surface soils and declines rapidly with depth. Culturable counts of heterotrophic bacteria (Balkwill et al., 1998; Kieft et al., 1998; Taylor et al., 2002), direct counts of total bacteria (Bundt et al., 2001; Ekelund et al., 2001; Horwath, 1993; Kieft et al., 1998; Taylor et al., 2002), total PLFA (Balkwill et al., 1998; Fierer et al., 2003b; Kieft et al., 1998), total DNA (Bundt et al., 2001; LaMontagne et al., 2003; Taylor et al., 2002), biomass by chloroform fumigation and extraction (Fierer, 2003; Horwath, 1993) or incubation (Horwath, 1993), and microbial abundance based on total glucose uptake (Kieft et al., 1998) or glucose mineralization (Kieft et al., 1998) all show a sharp decrease with depth through the vadose zone. However, culturable counts tend to increase in the capillary fringe (Konopka and Turco, 1991). Culturable counts are generally much lower than direct counts (e.g., Dodds et al., 1996), and the number of cultivatable cells declines more rapidly with depth than the total numbers of cells (Bachofen et al., 1998; Kieft et al., 1998). For example, subsurface counts of culturable heterotrophs were approximately 10% of overlying surface soils (Konopka and Turco, 1991). At the same sites, the densities of total bacterial cells (by direct counts) in deeper vadose materials were approximately 20% of those found in overlying soils (Konopka and Turco, 1991).

Analysis of previously published data sets indicates that soil microbial abundance, like soil C, can be modeled reasonably as a power function of depth (Eq. [1], Table 2). In Table 2, the mathematical functions are for the more extensive datasets that were found. In some cases, exponential functions also provided reasonable fits to the data (not shown), but the majority of the data sets were better described by power functions. The coefficients preceding the dependent variable (Table 2) are highly site, soil, and season-specific and depend on the largest microbial population density in the dataset (i.e., the density at the upper end of the depth interval). In general, population sizes do not change very much below the region of initial, rapid decline (Taylor et al., 2002) and often remain relatively constant until they increase within the capillary fringe (Konopka and Turco, 1991) and into the groundwater zone. Thus, the simple power function suggested in Eq. [1] may be descriptive of published data, but it probably overestimates the attenuation in microbial population size with increasing depth below the surface, thus underestimating the abundance of microbes in the subsurface. Again, this is similar to the application of Eq. [1] to C profiles where this function is most applicable to describing the rapid decline in relatively shallow (<3 m) depths.

In shallow vadose zones, the majority of the total microbial biomass can be found in near-surface layers and in the capillary fringe, where cell densities are highest. In vadose zones that extend many meters deep, a larger portion of the total microbial biomass resides below the surface layers. Assuming constant population densities with depth, a select number of cores and studies suggest the top 8 m of the terrestrial subsurface harbors an estimated 2.5×10^{29} prokaryotes and the subsurface region below 8 m (which includes saturated zones) contains approximately 2.5×10^{30} prokaryotes (Whitman et al., 1998). However, these estimates might be adjusted down if one takes into account the rapid decline with depth reported for all measures of microbial abundance in soils discussed above.

Nonbacterial biomass by PLFA, which includes plants, algae, diatoms and fungi, decreases more dramatically with depth than does bacterial biomass (Fierer et al., 2003b; Taylor et al., 2002). By assessing the relative changes in bacterial/fungal ratio, bacterial abundance is greater than fungal abundance at all depths in the vadose zone (Fierer et al., 2003b; Fritze et al., 2000). In some cases, the ratio of fungi to bacteria by PLFA declines with depth through the surface organic horizons

Table 1. Cultivation-dependent and independent methods for quantifying vadose zone microbial abundances. All methods have known advantages and disadvantages.

Methods	Citations	Advantages and disadvantages†
	Cultivation-dependent methods	
CFUs (aerobes)	Balkwill et al., 1998; Beloin et al., 1988; Bone and Balkwill, 1988; Brockman et al., 1992; Dodds et al., 1996; Ghiorse and Balkwill, 1983; Hickman and Novak, 1989; Kieft et al., 1993; Kieft et al., 1998; Taylor et al., 2002	A1: Isolates are available for further study and can be used to generate nucleic acid probes. D1: A small fraction of microbes in nature can be cultivated in the laboratory. D2: Anaerobes and some microaerophiles are excluded.
MPN (aerobes)	Beloin et al., 1988; Hickman and Novak, 1989	A2: Less material- and time-intensive than CFU enumeration on solid media. D1, D2
MPN (anaerobes)	Kieft et al., 1998	D3: Selects for planktonic growth when vadose microbes are usually attached A2 A3: Allows for enumeration of anaerobic populations. A4: Compared with solid media cultivation, easy to achieve and maintain O_2–free headspace. D1, D3
MPN (grams dry weight)	Beloin et al., 1988	A2 A5: Mass basis for reporting results D1, D2, D3
MPN (protozoa)	Ekelund et al., 2001	A2, D1, D3
SIR (expressed as C_{mic})	Fierer et al., 2003a; Fritze et al., 2000; Taylor et al., 2002	A6: Provides indication of how active microbes are in situ D1
Substrate mineralization or uptake	Kieft et al., 1998	A6 D1
	Cultivation-independent methods	
DNA	Bundt et al., 2001; LaMontagne et al., 2003; Taylor et al., 2002	A7: Captures culturable and unculturable populations. D4: DNA content per cell varies. D5: incomplete or biased recovery during extraction.
γ Proteobacteria	Balkwill et al., 1998	A7 A8: Specific to an important phylogenetic group. D5
α Proteobacteria	Balkwill et al., 1998	A7, A8, D5
Acridine orange (AO) direct counts	Beloin et al., 1988; Bone and Balkwill, 1988; Brockman et al., 1992; Dodds et al., 1996; Ekelund et al., 2001; Federle et al., 1990; Hickman and Novak, 1989; Kieft et al., 1993	A7 A9: Cell sizes can be estimated during microscopy. A10: specific to DNA D6: Can't completely extract cells. D7: Soils with high background fluorescence obscure visualization. D8: Does not distinguish viable from nonviable. D9: Not specific to prokaryotes.
DAPI direct counts	Bundt et al., 2001; Kieft et al., 1998; Taylor et al., 2002	A7, A9, A10 D6, D7, D8, D9
Fungi direct counts	Balkwill et al., 1998; Ekelund et al., 2001	A7, A9 D6, D7, D8
PLFA microbial biomass	Blume et al., 2002; Federle et al., 1990; Franzmann et al., 1998; Fritze et al., 2000; Kieft et al., 1998	A7 A11: Reproducible chemical extraction and quantification. A12: Group-specific quantification possible. A13: Metabolic status, e.g., starvation, indicated.

Continued next page.

Table 1. Continued.

Methods	Citations	Advantages and disadvantages†
		D8 D10: Variable profiles within populations. D11: Aggregated and not easily related to abundance of individual propagules.
Actinomycete PLFA	Fierer et al., 2003a	A7, A11, A12, A13 D8, D10, D11, D12
Bacterial PLFA	Blume et al., 2002; Fierer et al., 2003a; Fritze et al., 2000; Kieft et al., 1998	A7, A11, A12, A13 D8, D10, D11
Bacterial PLFA phospholipid-PO_4	Blume et al., 2002	A7, A11, A13 A14: Normalized reporting allows for intersite comparisons D8, D10, D11
Fungal PLFA	Fierer et al., 2003a; Fritze et al., 2000	A7, A11, A12, A13 D8, D10, D11
PLFA protozoa	Fierer et al., 2003a	A7, A11, A12, A13 D8, D10, D11
Fungal/bacterial abundance ratio	Fritze et al., 2000	A7, A11, A12, A13 D10, D8, D11
Trans-cis PLFA	Blume et al., 2002	A7, A11, A13 D8, D10, D11
DGFA (dead cells)	Kieft et al., 1998	A11, A12, A15: Accounts for dead biomass D10, D11
DGFA/PLFA (dead to live cells)	Kieft et al., 1998	A7, A11, A12, A13, A15 D10, D11
High or low G+C G^+	Balkwill et al., 1998	A7, A11, A12 D8, D11
Microbial C by fumigation/extraction	Bundt et al., 2001; Fierer et al., 2003a; Horwath, 1993	A7, A11, A14 D5, D8, D11
Organic C (mass/mass, or % basis)	Ajwa et al., 1998; Blume et al., 2002; Brockman et al., 1992; Bundt et al., 2001; Ekelund et al., 2001; Franzmann et al., 1998; Fredrickson et al., 1995; Konopka and Turco, 1991; Rovira and Vallejo, 1997; Wood and Petraitis, 1984	A7, A11, A14 D8, D11, D12: not specific to microorganisms; fresh or old plant-derived C can generate this signal.

† Abbreviations A1 and D1, etc. denote Advantage 1 and Disadvantage 1 and are repeated in subsequent cells to indicate that it applies to other methods as well.

Table 2. Power functions describing microbial abundance and organic C vs. depth (y).

Method	Submethod	Source	Depth interval	Function, $x = f(y)$†	R^2
			m		
Direct counts‡	AODC‡	Bone and Balkwill, 1988	0.03–3.0	$6E7y^{-0.9096}$	0.68
		Konopka and Turco, 1991	1.8–12.3	$2E8y^{-0.7625}$	0.98
	DAPI‡	Taylor et al., 2002	0.15–4.05	$2E9y^{-1.1759}$	0.89
		Kieft et al., 1998	0.05–38	$5739y^{-2.2171}$	0.68
		Kieft et al., 1998	0.05–12	$116157y^{-2.0685}$	0.67
		Bundt et al., 2001	0.05–0.76	$7E6y^{-0.6734}$	0.95
CFUs‡	PTYG§	Bone and Balkwill, 1988	0.03–3	$48761y^{-1.5965}$	0.99
	Dilute PTYG§	Bone and Balkwill, 1988	0.03–3	$78847y^{-1.6292}$	0.99
	SSA§	Bone and Balkwill, 1988	0.03–3	$152990y^{-1.5667}$	0.90
	Soil 1¶	Hickman and Novak, 1989	0.005–4.6	$108849y^{-0.5192}$	0.70
	Soil 2¶	Hickman and Novak, 1989	0.005–4.6	$706248y^{-0.248}$	0.91
	Soil 3¶	Hickman and Novak, 1989	0.005–4.6	$440702y^{-0.2155}$	0.85
	R2A§, Iowa clay¶	Taylor et al., 2002	0.15–2.85	$2E7y^{-0.7603}$	0.98
	SEA§, Iowa clay¶	Taylor et al., 2002	0.15–2.85	$2E7y^{-0.6227}$	0.99
	R2A§, Michigan sand¶	Taylor et al., 2002	0.15–4.05	$8E6y^{-0.8732}$	0.99
	SEA§, Michigan sand¶	Taylor et al., 2002	0.15–4.05	$7E6y^{-0.8883}$	0.95
Organic C g/kg‡		Konopka and Turco, 1991	0.35–18.3	$6.98y^{-0.7184}$	0.98
		Bundt et al., 2001	0.045–0.75	$3.961y^{-.6912}$	0.99
	TP¶	Ajwa et al., 1998	0.2–4.4	$2.7299y^{-1.0864}$	0.88
	Ag¶	Ajwa et al., 1998	0.2–5.1	$2.8128y^{-0.4348}$	0.63

† As explained in the text, a power function model (y = depth) provided a reasonable fit (by R^2 of least squares regression) for the majority of the data analyzed.
‡ See Table 1 for comments on methods.
§ Media formulation; refer to source publication.
¶ Soil name; refer to source publication.

and increases in the deeper mineral horizons (Fritze et al., 2000). Direct counts of fungal populations also decline with depth and at a more dramatic rate than do direct counts of bacteria (Taylor et al., 2002). Protozoan population densities also decline rapidly with depth below surface soils (Fierer et al., 2003b), which is attributable to the declining abundance of bacteria with depth (Ekelund et al., 2001). An additional factor in protozoan population densities is their need for aerobic conditions; in frequently saturated soils, even those rich in bacteria, protozoan populations are low (Ekelund et al., 2001). Thus, the abundance of bacteria is not a discrete predictor of protozoan population sizes. Still, the close relationship between bacterial population densities and protozoan population densities is predictable to the extent that it can be approximated with a log-log function (Dodds et al., 1996; Ekelund et al., 2001).

The abundance of microbes in the vadose zone varies more with depth through an individual profile than it varies among different soil profiles (Fritze et al., 2000). The depth-dependent distributions of microbes in a profile may be related to changes in physicochemical properties (Blume et al., 2002) with depth. For example, population sizes and activity of microbes appear to correlate with changes in soil texture through the vadose zone profile (Konopka and Turco, 1991). However, microbial biomass based on PLFA and culturable heterotrophs did not correlate with either water content or water potential (Balkwill et al., 1998), which are related to texture. Rather, there is a strong correlation between measurements of bacterial biomass and soil C contents across a range of soils that differ in texture. This relationship seems to hold true whether microbial biomass is estimated by direct counts, culturable counts (Palumbo et al., 1994; Severson et al., 1991; Veeh et al., 1996), SIR (Fritze et al., 2000), extracted DNA (LaMontagne et al., 2003; Taylor et al., 2002), total PLFA (Fritze et al., 2000), or by phospholipid-PO_4 (Blume et al., 2002). In general, the relationship between vadose zone population sizes and soil texture is a result of the effects of soil texture on soil C content. In fact, vadose zone profiles that are texturally and nutritionally uniform with depth harbor microbial populations that vary little with respect to their densities and community composition (Musslewhite et al., 2003). Increased energy, in the form of organic C for chemoheterotrophs, supports greater cell densities in surface soils. Just as organic C decreases rapidly with depth (Eq. [1]), so do soil microbes that mostly require organic C for growth.

The distribution of microbes as a function of vadose zone depth helps us mathematically model microbially mediated processes for the entire profile. However, microbial processes may also be influenced by the distribution of microbes at other spatial scales within a given vadose zone. One source of mesoscale variation is linked to the spatial distribution of bulk soil properties such as flow paths that preferentially conduct water through the vadose zone. In surface soil, microbial C, DNA, and bacterial direct counts have been shown to be higher in the vicinity of preferential flow paths, possibly reflecting the higher availability of organic C in these zones (Bundt et al., 2001). Even at this pore scale, further gradients exist—bacterial populations several microns away from pore walls in surface soils are higher than bacterial population sizes immediately along pore walls (Nunan et al., 2003), presumably due to the hostile effects of erosion and desiccation. However, in subsurface soil (below about 50 cm), the differences between population sizes in preferential flow paths vs. surrounding soils can be small (Bundt et al., 2001) or variable (Nunan et al., 2003). Nutrients (N and C, Table 2) decline dramatically with depth in both bulk soil and in the vicinity of preferential flow paths (Bundt et al., 2001); any increase in nutrient levels associated with the flow paths may not

be sufficient in magnitude to significantly enrich flow paths as compared with the surrounding matrix.

Compared with what we know about the distribution of vadose zone microbes at larger spatial scales, we know very little about microscale spatial distributions and their effect on the rate of biodegradation and other microbial processes in the vadose zone. At the centimeter scale, the population of aerobic culturable heterotrophs can vary by 10-fold in a single sample (Zhang et al., 1997). At the scale of individual soil microbes, there are at least three known possible configurations for microbial growth habits which may influence the spatial variability in microbial physiologies. Microbes can colonize the air–water interface (Wan et al., 1994), live freely in water, or attach to surfaces where they would grow as biofilms consisting of cells and their associated exopolymeric substances (EPS) (Cheshire, 1979; Oades, 1984). These conceptual configurations are mainly applicable to bacteria, the most abundant microbes in the vadose zone, but they may also describe the growth habits of other microbial particles, such as fungal spores, viral particles, and protozoa. The configuration of filamentous fungi is important at scales between the macro- and microscales because their hyphae can bridge pores and particles. The growth habit undoubtedly affects the movement of microbes through the vadose zone because microbes at the air–water interface (Wan et al., 1994) and in bulk water are mobile, but most soil microbes are embedded in EPS and are relatively immobile (Gammack et al., 1992). The growth habit also affects availability of nutrients to microbes and their susceptibility to predation. Microbes in EPS are relatively more protected from predation, but EPS can restrict nutrient diffusion (Holden et al., 1997b). Whether or not microbes live as a "biofilm" blanketing surfaces of vadose zone materials is open for debate (Else et al., 2003) since cells in C-depleted unsaturated porous media are observed to have few neighbors (Holden et al., 2002; Nunan et al., 2003).

Composition and diversity of vadose zone microbial communities

The composition of microbial communities in the vadose zone is strongly depth dependent. Using principal components analysis of PLFA signatures along two depth profiles (0–2 m), Fierer et al. (2003b) showed that the overall composition of microbial communities changes substantially with soil depth. The PLFA profiles of deeper soils also seem to have a higher degree of seasonal variability than surface soils (Blume et al., 2002). Perhaps seasonal changes in surface soil phospholipid fatty acid profiles are too small to observe because the surface soils are routinely exposed to large fluctuations in temperature and moisture (Blume et al., 2002; Fierer et al., 2003c). Consistently, an analysis of 16S rDNA genes by terminal restriction fragment length polymorphisms (TRFLPs) indicates that the depth dependency of vadose zone bacterial communities is stronger than the seasonality of surface soil communities (LaMontagne et al., 2003).

The PFLA profiles become more simple (Fierer et al., 2003b) and the numbers of TRFLP peaks decrease (LaMontagne et al., 2003) substantially with depth through vadose zone profiles, indicating a decline in species richness with depth. In addition, the overall level of bacterial diversity, calculated using the number and relative abundance of TRFLP peaks, is 75% lower in the subsurface layers than in the surface layers (LaMontagne et al., 2003). As another indicator of taxonomic diversity, surface soils have been shown to contain 16S rRNA gene sequences from twice as many phylogenetic divisions in the domain *Bacteria* as compared with subsurface soils (Zhou et al., 2004). Metabolic diversity measured with either Biolog plates (Kieft et al., 1998) or substrate profiling (Fierer, 2003) also declines with depth. The ratio of DGFA to PLFA increases with depth, indicating that the proportion of dead cells increases with depth below the surface, a possible explanation for the observed decrease in culturability with depth (Kieft et al., 1998). Still, bacteria in the deep subsurface appear remarkably diverse. For example, even in a 16- to 44-m deep vadose zone region contaminated with radioactive metals (^{137}Cs and ^{99}Tc) where culturable counts of bacteria were low, isolates were diverse and bacteria had adapted to survive 5 kGy of radiation (Fredrickson et al., 2004). The most radiation-resistant bacteria were high G+C, gram-positives from a variety of genera including relatives of *Deinococcus radiodurans* (Fredrickson et al., 2004).

The decrease in richness and diversity across the vadose zone depth profile, as evidenced by the range of methods described here, coupled with the known patterns of C with depth (e.g., Eq. [1]), suggests the vadose zone as an interesting test for the species-energy hypothesis (LaMontagne et al., 2003; Wright, 1983). An alternative explanation for surface soil microbial communities being relatively more diverse than deep soil communities is that low water content leads to spatial isolation of microbes in surface soils (Zhou et al., 2004). More likely, it is a combination of variable mass transfer in surface soils, fluctuating temperature and water stresses, and higher C and other nutrient concentrations that simultaneously select for more diverse microbial populations in surface as compared with subsurface soils.

Changes in community composition through the vadose zone profile can be attributed to shifts in the relative proportions of specific microbial populations and the presence of exotic populations at depth. For example, relative to gram-negative bacteria, gram-positive bacteria and actinomycetes become more abundant with depth (Blume et al., 2002; Fierer et al., 2003b). Terminal restriction fragment length polymorphism analyses suggest that specific groups of *Bacteria* are lost from the community with increasing depth: some operational taxonomic units (OTUs) found in the subsurface are also found in the surface, but many of the OTUs found in surface soils are absent at depth (LaMontagne et al., 2003). However, representatives of the *Archaea* can be grouped specifically depending on soil depth (Pesaro and Widmer, 2002). Subsurface soils contain Proteobacteria, gram-positive bacteria, and species of *Vario-*

vorax, *Pseudomonas*, and *Firmicutes* (Balkwill et al., 1997; LaMontagne et al., 2003) that have also been observed in surface soils.

Subsurface communities are likely partially composed of those microbial populations found in surface communities that are adapted to the low nutrient conditions found in the subsurface. Consistently, PLFA markers (ratios of cyclopropyl to monoeonic precursors, and total saturated to total monunsaturated fatty acids) suggest an increased level of C starvation with depth in the vadose zone (Blume et al., 2002; Fierer et al., 2003b). Also, intracellular inclusion bodies of polyphosphate or polyhydroxyalkanotate are typically found in microbes growing under nutrient-rich conditions, but are infrequently observed in vadose zone microbes (Bone and Balkwill, 1988). However, transmission electron microscopy has revealed that bacteria residing 4 m below the surface in an unsaturated zone have a thick (e.g., equal to the cell diameter) layer of EPS (Ghiorse and Balkwill, 1983). The presence of well-developed EPS layers may partly explain why bacteria become less extractable with depth (Taylor et al., 2002), but also suggests that C is at least abundant enough at depth for EPS production.

Groundwater is largely recharged by surface waters percolating through the vadose zone. This movement of water should also transport surface microbes and surface-derived nutrients deeper into the vadose zone. Therefore, seasonal precipitation and hydraulic recharge patterns should influence subsurface microbial community composition. Since the magnitude and frequency of recharge varies among sites, there should be corresponding site-level differences in the composition of vadose zone communities. In areas of high recharge, gram-positive bacteria are more abundant with depth; the opposite is found in low recharge environments (Balkwill et al., 1998). In areas of high recharge, subsurface gram-negative organisms show signs of stress, possibly attributable to the rapid shift experienced in moving from the surface to subsurface environment (Balkwill et al., 1998). Along the preferential flow paths that conduct recharge water, *Bacterial* communities differ from the surrounding soil, but the *Archaeal* and *Eukaryal* community fractions differ even more strongly (Bundt et al., 2001). Vadose zone microbial community composition is more constant with depth when there are more preferential flow paths, presumably because microbes are transported more extensively throughout the vadose zone (Balkwill et al., 1998). Overall, site hydrology has a strong influence on the distribution and diversity of microorganisms along the vadose zone profile (Balkwill et al., 1998). Unfortunately, we do not know enough about the distribution of specific microbial groups through the vadose zone to generate predictive mathematical models of depth-specific microbial processes in the vadose zone.

VADOSE ZONE MICROBIAL PROCESSES

While microbial population densities decrease substantially with depth, a large fraction of the total vadose zone biomass exists in deep soils (e.g., Fierer et al., 2003b). The microbes residing in deeper portions of the vadose zone can intercept or immobilize toxic pollutants and alter pollutant forms into less harmful metabolites. In fact, leaving waste in place in the vadose zone to "naturally attenuate" is predicated on subsurface microbes catalyzing useful reactions. What evidence do we have that vadose zone microbes are active in situ and able to respond to substrate additions? More importantly, do we know enough about microbial processes in the vadose zone to build predictive models? The existing body of literature begins to answer the former question. The answer to the latter question is probably "no, we do not yet know enough about microbial processes in the vadose zone to build predictive models." Areas for improvement are suggested by examining and looking beyond the existing body of knowledge.

Metabolic Potential through the Profile

As mentioned above, microbial abundances decrease with depth through the vadose zone. Not surprisingly, we see a similar pattern when we examine rates of microbial activities per mass of soil. Dehydrogenase activity (Taylor et al., 2002), β-galactosidase induction (Kieft et al., 1998), CO_2 evolution from substrate induced respiration or SIR (Ajwa et al., 1998; Chapatwala et al., 1996; Fierer, 2003; Horwath, 1993), N mineralization (Ajwa et al., 1998; Horwath, 1993), incorporation of $^{32}PO_4$ (Konopka and Turco, 1991), and FDA hydrolysis (Federle et al., 1986) all decrease with depth through the vadose zone. The activities of enzymes involved in C, N, P, and S transformations show similar patterns with depth (Taylor et al., 2002). The decrease in the rates of microbial activity is primarily a result of the parallel decrease in microbial abundance and substrate availability with vadose zone depth (Federle et al., 1986; Swensen and Bakken, 1998; Taylor et al., 2002). However, respiration rates in deeper soils can be as high or higher than in surface soils when either water or aqueous phase nutrients are added (Chapatwala et al., 1996; Dick et al., 2000). In fact, the specific activity (activity per cell) may actually increase at intermediate or greater depths (Blume et al., 2002). The specific activity of microbes appears to be affected by pore size. Microbes in small pores (<0.2 μm) appear inactive in situ compared with microbes in larger, interconnected pores (Fredrickson et al., 1997), probably because the diffusional resupply of nutrients is restricted in small pores. In contrast, larger, interconnected pores tend to contain more metabolically active microbes (Fredrickson et al., 1997). Less is known regarding the effects of temperature on microbial activity in the vadose zone, but ^{3}H-acetate incorporation was shown to be higher in warm vs. cold seasons for all depths in one study (Blume et al., 2002).

Carbon and Nitrogen Processing in the Vadose Zone

The concentration of CO_2 vs. depth is used to indicate microbial mineralization of native C, that is, microbial respiration in the vadose zone. In a sandy vadose zone, CO_2 content varied with depth from 0.04 to 1.29% (Hendry et al., 1999). Carbon dioxide concentration increases

from 0.035% aboveground to up to 100-fold (approximately 4% or more) with depth (Bacon and Keller, 1998; Davidson and Trumbore, 1995; Fang and Moncrieff, 1998; Keller and Bacon, 1998; Richter and Markewitz, 1995; Wood et al., 1993), which contributes to the increased alkalinity in deeper soils (see Environmental Factors above). The increase in CO_2 concentrations with depth results from ongoing, albeit low, rates of biotic CO_2 production and low rates of belowground diffusion (Davidson and Trumbore, 1995; Richter and Markewitz, 1995). The diffusional constraints on CO_2 evolution in shallow (≈1-m depth) (Fang and Moncrieff, 1998) and deeper (to ≈8 m) (Davidson and Trumbore, 1995) profiles are demonstrated by a near linear relationship between CO_2 concentrations and soil depth. High soil gas CO_2 concentrations in deep soil can either be from plant or microbial respiration (Hendry et al., 1999). Plant roots are often the largest source of CO_2 at depth (Davidson and Trumbore, 1995; Hendry et al., 1999), but microbial respiration can also be a significant source of CO_2 in deep vadose zone layers where roots are largely absent (Keller and Bacon, 1998; Lee et al., 2003; Wood et al., 1993).

Annual trends in vadose zone CO_2 concentrations vary widely depending on vegetation type and climate, but some generalizations can be made. In situ respiration as indicated by CO_2 evolution at intervals along the depth profile (Hirsch et al., 2002; Risk et al., 2002) and respiration in laboratory incubations (Chapatwala et al., 1996) increase with increasing temperature. During the growing season, respiration appears greater in surface vs. subsurface soils (Keller and Bacon, 1998) because respiration in surface soils has increased (Hendry et al., 1999). During the winter, CO_2 concentrations at depth are observed to be higher (Hendry et al., 1999) partly because high CO_2 concentrations persist at depth. Drying–rewetting cycles in surface soils stress microbes (Fierer et al., 2003c), and this stress is manifested in lower C and N mineralization rates as compared with unstressed subsurface microbes amended with the same substrates (Rovira and Vallejo, 1997). However, the high OM content that occurs in surface soils helps reduce the impact of low water on soil microbial activity (Rovira and Vallejo, 1997). Overall, seasonal patterns in CO_2 concentrations at depth are largely a function of diffusion rates, C availability, and plant phenology.

A strong inverse correlation between vadose zone thickness and water table dissolved organic C (DOC) concentrations indicates that DOC is strongly attenuated in the vadose zone (Pabich et al., 2001) as a result of either physical sorption onto mineral surfaces or microbial mineralization. While CO_2 can be produced by abiotic processes (such as carbonate dissolution), the ^{13}C signatures of vadose zone CO_2 suggest that the CO_2 is generally of biogenic origin (Amundson et al., 1998; Wood et al., 1993). Carbon-14 isotopic analysis of profile CO_2 suggests that vadose zone CO_2 is generally derived from organic C sources that are relatively young (1–50 yr; Trumbore, 2000). However, older organic C (100–10 000 yr) may also be significant sources of vadose zone CO_2 (Bacon and Keller, 1998; Hirsch et al., 2002; Keller and Bacon, 1998; Trumbore et al., 1995).

Microbes in the vadose zone, down to approximately 8 m, are able to immobilize NH_4^+ or NO_3^- as well as mineralize organic N, but the availability of C appears to limit immobilization (Dick et al., 2000). In general, comparatively little information is available regarding N mineralization in the vadose zone.

Kinetics of C and N Mineralization

Studies of CO_2 concentrations and isotopic composition confirm that microbial processing of organic C occurs in the vadose zone, but how fast? Mineralization rates measured in laboratory incubations of vadose material suggest the processes are first order, whether C is exogenous or naturally occurring (Table 3). Glucose mineralization potential was first order in laboratory incubations of vadose material, and rates declined with depth (Konopka and Turco, 1991) (Table 3). However, first-order rate constants for mineralization of native C and N in the vadose zone did not correlate with either depth or initial soil C content in tallgrass prairie or agricultural soils (Ajwa et al., 1998), but the soils were preincubated at warm temperatures for a week before mineralization studies, which may have substantially altered microbial community composition, C and N content or quality, or all of these factors. Using a modification of Bonde and Rosswall's double exponential model (Bonde and Rosswall, 1987) of C mineralization in soil incubations, Horwath (Horwath, 1993) found that first-order rate constants for the labile C pool were higher below 25 cm (to 1 m) than above 25 cm. This is similar to the report of Ajwa et al. (1998), wherein first-order native C mineralization rate constants increased between depths of 0.2 to 0.8 m below ground, decreased at 2.6 m, and then increased again steadily until the water table depth at approximately 5 m (Table 3).

Nitrogen mineralization in Horwath's studies was zero order for all soils to 1 m, and the surface rate was approximately five times higher than the subsurface rate (Horwath, 1993). As an alternative to zero- or first-order models, N mineralization along the vadose zone profile may better fit a "consecutive reaction" model (Ellert and Bettany, 1988) that includes terms for the original source, mineralization, and the formation of intermediates (Ajwa et al., 1998). Using either the consecutive-order or first-order model, Ajwa et al. (1998) found that N mineralization rate constants decreased with depth overall but without regularity (Ajwa et al., 1998), and there was no significant correlation between C and N first-order mineralization rate constants.

Denitrification in the Vadose Zone

A significant amount of attention has focused on denitrification in the vadose zone, largely because of its importance in reducing the levels of NO_3^- entering the groundwater. The rate of heterotrophic denitrification is largely determined by the abundance of denitrifying bacteria, the quantities of organic C and NO_3^-, and the extent of anaerobiosis (Paul and Clark, 1989). A number

Table 3. Carbon mineralization rate constants by depth, site, substrate, temperature, and moisture in the vadose zone.

Depth	Site	Substrate	C_o	T	Moisture	k	Source
m				°C		d^{-1}	
0–0.25	poplar forest	labeled SOM	112.3 μg C g^{-1}	25	15.4%	0.04	Horwath, 1993
0.25–0.60			25.9 μg C g^{-1}			0.04	
0.60–1			29.1 μg C g^{-1}			0.09	
0.35	agricultural	glucose	10 μ*M*	12	slurry	1.34	Konopka and Turco, 1991
1.8		glucose	10 μ*M*			0.23	
6.3		glucose	10 μ*M*			0.036	
9.3		glucose	10 μ*M*			0.06	
12.3		glucose	10 μ*M*			0.09	
0.2	tallgrass prairie	SOM	2.561 g kg^{-1}	35	WHC	0.0042	Ajwa et al., 1998
0.8			0.496 g kg^{-1}	35		0.0072	
2			0.43 g kg^{-1}	35		0.0031	
3.2			0.57 g kg^{-1}	35		0.0027	
4.4			0.265 g kg^{-1}	35		0.0064	
0.2	agriculture	SOM	1.655	35	WHC	0.0029	Ajwa et al., 1998
0.8			0.363	35		0.0059	
2			0.004	35		0.0040	
3.3			0.0018	35		0.0018	
3.9			0.0038	35		0.0038	
5.1			0.0105	35		0.0105	

of studies suggest that denitrification can occur at significant rates in even the deepest layers of the vadose zone. Nitrogen-15 natural abundance signatures of soil gas in the vadose zone (≈10 m deep) and the abundance of denitrifying organisms indicate that the rate of denitrification below recharge basins can be significant (Fryar et al., 2000). In laboratory incubations, evolution of N_2O from vadose samples assayed for denitrification potential is not immediate and instead shows a significant lag phase (Paramasivam et al., 1999; Sotomayor and Rice, 1996). Further, denitrification rates do not decline continuously with depth, and there is a considerable level of microsite variability (Artiola, 1997; Paramasivam et al., 1999; Parkin, 1987; Sotomayor and Rice, 1996). However, in general, denitrification rates decrease sharply with depth below the surface, by approximately 10- to 100-fold for a depth interval of 0 to between 10 and 30 cm (Luo et al., 1998) in one study and by 50 to 100% between the surface and 90 cm (Paramasivam et al., 1999) in another. The magnitude of the decrease in denitrification rates through the vadose zone profile can be affected by surface soil management practices (Artiola, 1997; Sotomayor and Rice, 1996). Denitrification decreased by a factor of 10 000 for a depth interval of 0 to 14 m beneath fertilized soil, but decreased by a factor of 100 over the same depth interval under sewage sludge–amended soil (Artiola, 1997). The low rates of denitrification at deeper vadose zone depths can be attributed to a variety of factors, including: low denitrifier populations (Cannavo et al., 2002; Luo et al., 1998; Paramasivam et al., 1999), low availability of organic C (Artiola, 1997; Cannavo et al., 2002; Luo et al., 1998), and low availability of NO_3^-–N (Paramasivam et al., 1999). The addition of C and N appears to stimulate denitrification in vadose material more than the addition of NO_3^- alone (Artiola, 1997; Sotomayor and Rice, 1996), and the type of C appears to have a strong influence on denitrification rates in the vadose zone (Artiola, 1997).

Denitrifying bacteria are present at depths >1 m below the surface, but their specific denitrification potential (per cell denitrification rates under nonlimiting C and N conditions) appears to decrease significantly over a depth interval from the surface to approximately 1.5 m due to shifts in either denitrifier community composition or physiology with depth (Cannavo et al., 2002). However, a strong positive correlation between denitrification potential and total heterotrophs implies that denitrification in the vadose zone may be performed by aerobic heterotrophs that denitrify when conditions are favorable (Sotomayor and Rice, 1996). Thus, the denitrifier community may not be a distinct portion of the total microbial community found in the vadose zone. There was one report of abundances of NH_3-oxidizing bacteria and NO_3-reducing bacteria in the same samples (Whitelaw and Rees, 1980). At a grassland site in that study, the population sizes of NH_3-oxidizers and NO_3-reducers showed exact opposite trends with depth down to approximately 10 m below the soil surface (Whitelaw and Rees, 1980). However, at a fertilized site, NO_3-reducers were not detectable above the 30-m depth and down to 51 m were of similar magnitude and trend as NH_3-oxidizing populations (Whitelaw and Rees, 1980). The effects of the site (grassland vs. fertilized agricultural) per se are not clear because in another study denitrifiers were more abundant in the vadose zone under cultivated soils than under prairie soils (Sotomayor and Rice, 1996). This difference may punctuate the point that denitrification is, after all, driven by bacterial populations, nutrient conditions, and moisture, which may not predictably change with surface soil management.

Transformation of Pollutants

A number of lines of evidence suggest that vadose zone microbes can intercept and use pollutants as substrates. For example, the activity and biomass of microbes are often higher in vadose materials that have been contaminated with organic pollutants (Fredrickson et al., 1993). Since in situ natural bioattenuation is a com-

mon remediation strategy for a range of pollutants, the processes of pollutant biodegradation in the vadose zone are of considerable societal importance.

Pesticides

While pesticides are frequently detected in groundwater underlying agricultural fields with long application histories, the fact that concentrations are sometimes very low (Albrechtsen et al., 2001) or not detectable (Jacobsen et al., 2001) suggests that the microbial attenuation of pesticide residues may occur in the unsaturated subsurface. In soil and subsoils, the fates of pesticides may include sorption, volatilization, biodegradation, and abiotic transformation. In studies where the parent pesticide is not ^{14}C-labeled, it is difficult to infer biodegradation processes from the time course of the remaining parent compound because of potentially confounding sorption processes. For example, carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is removed in surface and subsurface soils (Karpouzas et al., 2001), but degradation can appear comparatively greater in the subsurface if sorption prevents extraction of the parent compound from surface soils. In this review, we largely focus on direct evidence for biodegradation and refer to abiotic fates of pesticides when necessary.

Under natural conditions, the attenuation of metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide], a broadleaf and annual grass herbicide, by sorption, volatilization, or biodegradation occurs at very low rates (Rice et al., 2002) or not at all (Konopka and Turco, 1991; Miller et al., 1997) in soils below the surface. However, moisture addition facilitated the biodegradation of metolachlor in deep soils, reducing the half-life of the parent compound from 289 d to 173 d under aerobic conditions at 25°C (Rice et al., 2002). Moisture also appeared to facilitate the removal of isoproturon {*N,N*-dimethyl-*N′*-[4-(1-methylethyl)phenyl]urea} from subsoils (Johnson et al., 2000). Decreasing mineralization of phenylurea herbicides, including isoproturon, occurs with increasing depth below surface soils (Issa and Wood, 1999; Sorensen et al., 2003), but mineralization of isoproturon can also be low in surface soils (Larsen et al., 2000). Mecoprop [(±)-2-(4-chloro-2-methylphenoxy)propanoic acid], a phenoxyacid herbicide, is mineralized in surface and subsurface soils (Larsen et al., 2000), but similarly to isoproturon, the amount of mineralization is twice as high in the surface than in the subsurface (≈1 m deep) (Larsen et al., 2000) (Table 4). Mecoprop mineralization, expressed as first-order rate constants for ^{14}C-CO_2 evolution (Larsen et al., 2000) or total mineralized compound (Vinther et al., 2001) correlates positively with organic C content and declines with depth. Acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide] is reported to mineralize below the 30-cm depth, but to only one-half the extent (≈13% below 30 cm vs. ≈27% above) of what occurs in surface soils (Mills et al., 2001). Similarly, alachlor degradation rates are mostly slower in deep vs. surface soils. However, some subsurface rates appear higher than surface rates (Pothuluri et al., 1990) which, on closer inspection, can likely be explained by higher subsurface culturable microbial populations. In fact there is a significant linear relationship between microbial population size and degradation rates for the aerobic data of Pothuluri et al. (Pothuluri et al., 1990). A similar relationship can be found in the broader biodegradation literature where *tert*-butyl alcohol biodegradation rate constants were linearly related to culturable bacteria (Hickman and Novak, 1989).

The microbial mineralization of atrazine [6-chloro-*N*-ethyl-*N′*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], a triazine herbicide, occurs at very low rates in the vadose zone, or not at all (Konopka and Turco, 1991; Larsen et al., 2000; Miller et al., 1997; Rodriguez and Harkin, 1997; Stolpe and Shea, 1995; Vanderheyden et al., 1997; Willems et al., 1996; Wood et al., 2002). Low but measurable rates of atrazine mineralization reported between 40 to 70 cm below the surface (Wood et al., 2002) were within the range of mineralization attributed to impurities in ^{14}C-labeled atrazine (Larsen et al., 2000). High mineralization (up to 30% of initial radioactivity after 20 d) observed in the subsurface (≈5 m) was observed to correlate with coarse textures and high microbial abundances (Vanderheyden et al., 1997). The number of microbes capable of degradation may be the most important factor governing atrazine mineralization in subsurface soils (Radosevich et al., 1996).

The typically low rates of atrazine mineralization may also be caused by low bioavailability of OM-sorbed atrazine, since there is often a negative correlation between atrazine mineralization and OM percentage in the vadose zone (Jacobsen et al., 2001; Radosevich et al., 1996). However, OM supports cometabolic activity that is characteristic of atrazine mineralization processes (Willems et al., 1996). As such, higher concentrations of OM in the vadose zone may increase the rates of atrazine degradation by either increasing microbial populations overall or by serving as a cosubstrate for cometabolizing degraders (Issa and Wood, 1999). Alternatively, where OM concentrations are low, but clay content is high, clay-mediated sorption may limit bioavailability (Konopka and Turco, 1991). Where clay content is intermediate, the availability of OM, as either a primary C source or determinant of high microbial population sizes, correlates positively with atrazine mineralization (Stolpe and Shea, 1995; Willems et al., 1996).

Dealkylation of atrazine to deethylatrazine (DEAT) occurs in subsurface soils with enough OM to support larger microbial populations (Rodriguez and Harkin, 1997), but partial degradation may do little to reduce the toxicity of atrazine. Also, despite the strong tendency for atrazine to sorb to OM or to clays in surface soils, atrazine is strongly mobilized during flooding events into subsurface soils (Kelly and Wilson, 2000) and may leach into groundwater where mineralization may (Ames and Hoyle, 1999) or may not (Rodriguez and Harkin, 1997) take place.

Mineralization of 2,4-D [(2,4-dichlorophenoxy)acetic acid], another widely used herbicide, significantly diminishes with depth along the vadose zone profile, apparently due to the declining size of biodegrading popula-

Table 4. Mineralization rate constants for pesticides in vadose materials.

Depth or depth interval	Description	Substrate	C_o	T	Moisture	Order	$-k$†	Source
m				°C				
0–0.3	Nicollet–Webster complex; previously unexposed	metolachlor	9 μg g^{-1}	25	−33 kPa	first	0.0086	Rice et al., 2002
0.9–1.2		metolachlor	9 μg g^{-1}	25	−33 kPa	first	0.0024‡	
0–0.3	Nicollet–Webster complex; previously unexposed	metolachlor	9 μg g^{-1}	25	saturated	first	0.0130	Rice et al., 2002
0.9–1.2		metolachlor	9 μg g^{-1}	25	saturated	first	0.0076	
0.06	unexposed farmland	mecoprop	2.2 μg kg^{-1}	10	WHC	first	−0.0365§	Larsen et al., 2000
0.3							−0.0248	
1.7							−0.0050	
0–0.25	sandy loam, planted in ryegrass	R-atrazine¶	200 μg kg^{-1}	20	40% WHC	zero	0.05 μg/g d	Willems et al., 1996
0.25–0.5	sandy loam	R-atrazine					0.029	
0.5–0.75		R-atrazine					0.022	
0.75–1.0		R-atrazine					0.029	
1.0–1.25		R-atrazine					0.007	
1.25–1.5	loamy sand	R-atrazine					0.024	
0	Huntington, Rossburg, and Nolin silt loams	R-atrazine	24 mg kg^{-1}	25	moist	first	0.204	Radosevich et al., 1996
1.7		R-atrazine	24 mg kg^{-1}				0.033	
2.3		R-atrazine	24 mg kg^{-1}				0.04	
5.8		R-atrazine	24 mg kg^{-1}				0.016	
0	Huntington, Rossburg, and Nolin silt loams	C-atrazine	24 mg kg^{-1}	25	moist	first	0.084	Radosevich et al., 1996
1.7		C-atrazine	24 mg kg^{-1}				0.03	
2.3		C-atrazine	24 mg kg^{-1}				0.0086	
0–0.25	sandy loam, planted in ryegrass	2, 4-D	30 μg kg^{-1}	20	40% WHC	first	4.9	Willems et al., 1996
0.25–0.5	sandy loam						2.5	
0.5–0.75							1.6	
0.75–1.0							1.1	
1.0–1.25							1.1	
1.25–1.5	loamy sand						14.7	
0–0.1	Cuombe Series brown calcareous, unexposed	2, 4-D	20 mg kg^{-1}	20	WHC	zero	4.15#	Shaw and Burns, 1998
0.1–0.2							4.84	
0.2–0.3							2.74	
0.3–0.4							3.65	
0.4–0.5							1.37	
0–0.3	Amsterdam silt loam	2, 4-D	380 μg kg^{-1}	24	33%	first	0.3048	Veeh et al., 1996
0.3–0.6							0.1848	
0.6–1.2							0.0528	
0–0.3	Haverson silty clay loam	2, 4-D	380 μg kg^{-1}	24	33%	first	0.264	Veeh et al., 1996
0.3–0.6							0.0984	
0.6–1.2							0.0744	

† Units for k are d^{-1} for first-order models, and μg/g d for zero-order models.
‡ Sterile controls exhibited the same performance as nonsterile treatments.
§ Rate constant for the evolution of ^{14}C-CO_2, hence the positive sign.
¶ R and C preceding atrazine are ring, and chain-labeled, respectively.
# Rate constant reported in percent per day.

tions and not due to sorption or other nutrient constraints (Shaw and Burns, 1998). 2,4-D mineralization rates in vadose zone materials appear to follow Michaelis–Menten kinetics and are enhanced by higher moisture content (Willems et al., 1996). Higher levels of organic C and higher microbial abundances, as occur in surface soils, correspond to higher rates of 2,4-D degradation relative to deeper soils (Veeh et al., 1996). However, the addition of an exogenous C source to subsurface soils inhibited the short-term rates of 2,4-D biodegradation (Shaw and Burns, 1998; Willems et al., 1996). Apparently, adding simple C substrates to subsoils may enhance 2,4-D degradation in the long term by increasing microbial abundances. Carbon substrate addition may decrease short-term 2,4-D degradation rates because simple C substrates are preferentially metabolized by vadose zone microbes. Interestingly, in one study where 2,4-D mineralization rates were high, rates were reported to be faster in subsurface (≈1.5 m) vs. surface soils (Willems et al., 1996). However, there was no obvious explanation for this observation and, unlike other studies where biodegradation and microbial biomass were highly correlated (e.g., Hickman and Novak, 1989; Pothuluri et al., 1990), there were no correlations between 2,4-D mineralization rates and either cell density or organic C (Willems et al., 1996).

The physicochemical properties of pesticides, such as the rate of partitioning into octanol vs. water (e.g., for atrazine or mecoprop), will effect pesticide bioavailability by altering sorption. Rapid or extensive biodegradation is predicated on pollutant bioavailability as well as a population of organisms capable of biodegradation (Konopka and Turco, 1991). The presence of a degrading population cannot be inferred from the size of the total heterotrophic population (Wood et al., 2002). Selection for pesticide-degrading microbes may occur in vadose materials underlying continuously treated soil (Pivetz and Steenhuis, 1995). But short lag phases pre-

ceding mineralization can occur in subsoils below topsoils with no known history of mecoprop, isoproturon, and atrazine application (Larsen et al., 2000). Also, soils with a long history of pesticide application can overlie subsoils with little atrazine or metaolachlor biodegradation potential if the compounds are never transported to the subsurface layers (Konopka and Turco, 1991). Biodegradation of 2,4-D is faster in macropores (Pivetz and Steenhuis, 1995) where the increased supply of nutrients sustains large populations of pesticide degraders. The spatial co-occurrence of catalysts and substrates, in this case bacteria and pesticides, is a strong determinant of biodegradation in the vadose zone.

Temperature and temperature fluctuations vary along the vadose zone depth profile. Biodegradation kinetics are highly temperature dependent (e.g., Table 4), and temperature can influence the rate expression used to describe pesticide biodegradation in the vadose zone (Radosevich et al., 1996; Veeh et al., 1996). For example, the biodegradation of atrazine in subsurface soils may appear to be first order at 25°C, but zero order at 10°C (Radosevich et al., 1996). Additionally, biodegradation kinetics (e.g., for alachlor) that appear to be first order in surface soils may be zero order in the deeper subsurface layers (Pothuluri et al., 1990), but this could be due to saturation of the subsurface soils with substrate, in this case alachlor. For pesticides such as mecoprop, bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide], and ethylene thiourea [4,5-dihydroimidazole-2(3H)-thione], the rates of mineralization in subsurface layers were most appropriately modeled as growth-associated processes while mineralization in surface layers appeared to be nongrowth related or cometabolic (Fomsgaard, 1997). The lag time preceding the onset of pesticide mineralization is often longer in subsurface soils than in surface soils (e.g., Fomsgaard, 1997; Radosevich et al., 1996; Shaw and Burns, 1998; Veeh et al., 1996), suggesting that the rate of enzyme induction (or the rate of microbial growth) varies as a function of vadose zone depth (Alexander, 1999). Logistic models better described the lag phase and S-shaped ^{14}C-CO_2 evolution curves associated with 2,4-D degradation in subsoils (Veeh et al., 1996). However, a first-order model provides a reasonable prediction of 2,4-D mineralization in subsurface soils. Since many pesticide biodegradation studies assume first-order kinetics, rate constants can be compared across studies (e.g., Veeh et al., 1996). For 2,4-D mineralization, kinetics were reasonably first order in the surface and at depth, and the half-lives decreased exponentially with depth (Veeh et al., 1996). However, as summarized in Table 4, only four of the seven studies exhibiting first-order kinetics show a significant exponential relationship between pesticide half-life and depth. For the others, either a linear or power function provides a better description of the decrease in biodegradation rates with depth below the surface. Overall, there is a paucity of data describing pesticide biodegradation kinetics in the vadose zone. To adequately understand pesticide biodegradation in the vadose zone, we need to empirically determine the appropriate functional expressions that describe pesticide mineralization across a wide spectrum of vadose zone depths, pesticide types, soil types, and environmental conditions.

Hydrocarbons

Hydrocarbon contamination in the vadose zone is widespread due to surface spills and leaking underground fuel tanks. In surface soils, the potential for petroleum biodegradation is nearly ubiquitous (Alexander, 1999), presumably because the intermediate products of plant decomposition are chemically similar to hydrocarbons (Dagley, 1975). In deeper soils, evidence is required on a case-by-case basis to show that biodegradation occurs either intrinsically (i.e., without addition of exogeneous nutrients or terminal electron acceptor) or with engineering (e.g., during bioventing of fuels). Evidence for petroleum biodegradation in the vadose zone comes from the concentration profiles of O_2 (Conrad et al., 1999; Franzmann et al., 2002; Ostendorf and Kampbell, 1991), CO_2 (Conrad et al., 1999; Franzmann et al., 2002), and volatilized hydrocarbon (Franzmann et al., 2002; Ostendorf and Kampbell, 1991) in soil gas. Evidence for active bioremediation also can be gleaned from the composition of the microbial community at the contaminated site. Within a shallow, JP-4-contaminated subsurface zone (vadose, capillary fringe and groundwater), the microbial community shifted across the contamination gradient (Stephen et al., 1999). The relative abundances of specific hydrocarbons can also provide evidence for active biodegradation. A relatively nondegradable hydrocarbon, MTBE, was found in groundwater beneath a leaking underground fuel tank while highly biodegradable aromatic hydrocarbons (e.g., BTEX) appear to be completely attenuated within the vadose zone (McLinn and Rehm, 1997).

Hydrocarbon concentrations are often below detection at the soil surface, where O_2 is plentiful. In subsurface soils, hydrocarbon concentration profiles appear linear when O_2 and CO_2 concentrations are consistently low and high, respectively (Conrad et al., 1999; Franzmann et al., 2002; McLinn and Rehm, 1997). The trends are strongly seasonal. In wet weather, water-filled soil pores limit O_2 diffusion to the aerobic petroleum-degrading bacteria, resulting in higher concentrations of hydrocarbons at depth (Franzmann et al., 2002). A shallow vadose zone (1 m varying seasonally to the surface) and heavily contaminated subsurface (1000 mg kg^{-1} total petroleum hydrocarbon) led to CH_4 production near the water table, which promoted strongly seasonal methanotrophic oxidation of methane in the unsaturated zone (Conrad et al., 1999). Both stable isotope (Aelion et al., 1997; Conrad et al., 1999; Hinchee and Arthur, 1991) and radioisotope (Aelion et al., 1997; Conrad et al., 1999) analyses of soil gases (CH_4 and CO_2) distinguishes gases that are derived from the mineralization of petroleum vs. the mineralization of soil OM (Aelion et al., 1997; Conrad et al., 1999; Hinchee and Arthur, 1991).

Under laboratory conditions, addition of inorganic nutrients and slurrying is necessary to observe toluene biodegradation in vadose materials taken from gasoline-

Table 5. Aerobic biodegradation kinetic parameters for hydrocarbons in vadose materials.

Depth	Conditions	Substrate	C_o	T	Moisture	Order	$-k$†	X‡	Source
m				°C			d^{-1}		
15	uncontaminated vadose materials, incubated in the laboratory, rate coefficients estimated for gas phase substrate.	Toluene	20 μg L^{-1}	28	13.7%	first	0.0576	1e7	Fuller et al., 1995
15		TCE	1 μg L^{-1}	28	13.7%	first	0.0312	1e7	
1	uncontaminated sand lysimeter; rate coefficients estimated for gas phase substrate.	*n*-hexane	48.1	20	5%	first	0.4	6e8	Pasteris et al., 2002
		n-octane	5.14				5.0		
		n-decane	1.27				5.0		
		n-dodecane	0.09				2.5		
		methyl-cyclo-pentane	37.4				0.15		
		methyl-cyclo-hexane	18.7				1.2		
		cyclohexane	26.1				0.8		
		isooctane	30.3				0.15		
		toluene	3.74				3.2		
Surface	gasoline-contaminated field sample incubated in lab and assayed for ^{14}C-CO_2	benzene	0.218 mg g^{-1}	22	wet	first	0.0267	nr§	Franzmann et al., 1999
0.25–0.35							0.0630		
0.5–0.6							0.0096		
2.24	Gasoline-contaminated site, rates modeled from in situ gases	BTEX	0.000784 g cm^{-3}	23	5%	first	257	nr	Lahvis et al., 1999
2.89			1.04 g cm^{-3}	23	10%		0.772		

† First-order rate constant.
‡ Total bacteria per gram dry weight.
§ Not reported.

contaminated subsurface environments down to 25 m (Fuller et al., 1995; Holden et al., 2001). This implies that toluene biodegradation may be strongly nutrient-limited in the deeper layers of the vadose zone (Fuller et al., 1995), but slurrying also restricts diffusional resupply of O_2, which could facilitate toluene removal by microaerobic vadose bacteria (Holden et al., 2001). In other studies, addition of inorganic nutrients appears to inhibit biodegradation in surface soils (Morgan and Watkinson, 1992). One explanation is that low solute water potential, which is known to inhibit toluene biodegradation (Holden et al., 1997a), can unintentionally result when fertilizer is added to enhance bioremediation (Braddock et al., 1997).

The kinetics of hydrocarbon biodegradation appear to be first order when biodegradation rates are estimated using field lysimeters (Pasteris et al., 2002), laboratory incubations of vadose materials (Fuller et al., 1995; Moyer et al., 1996), or in situ soil gas concentrations (Lahvis et al., 1999). Biodegradation rates are highly compound specific within the broad category of hydrocarbons (Moyer et al., 1996). First-order depletion coefficients for headspace toluene appear to range widely (Table 5), but the first-order coefficients reported by Fuller et al. (1995) and Pasteris et al. (2002) are nearly identical when calculated per unit bacterial biomass. Positive correlations between culturable bacterial population sizes and first-order biodegradation rate constants for other pollutants have been reported (Hickman and Novak, 1989). However, biodegradation rates of pollutants at the surface vs. at the 1-m depth vary with pollutant and soil type. Rates in the surface normalized to biomass can be anywhere from 1/20th to 20 times the rate at 1 m (Franzmann et al., 1998). Thus, microbial biomass is not necessarily the best predictor of hydrocarbon biodegradation rates in the vadose zone (Franzmann et al., 1998).

Hydrocarbon biodegradation rate constants estimated from numerical simulations of in situ vadose zone gas concentrations (Lahvis et al., 1999) are often higher than the constants estimated from laboratory-based studies (Moyer et al., 1996) (Table 5). There are two possible reasons for this: the rates of hydrocarbon biodegradation may not be truly first order, or the high degree of spatial heterogeneity in the vadose zone may make it difficult to accurately estimate soil gas concentrations (Moyer et al., 1996). Intrinsic fuel biodegradation in the vadose zone can also be modeled as a mixed- or zero-order process, depending on the concentration of limiting nutrients. When modeled as a first-order process, intrinsic biodegradation may appear faster (≈50 mg/kg d) than under bioventing conditions (≈5 mg/kg d) (Franzmann et al., 2002). However, first-order rate constants are generally higher when soils are biovented (Moyer et al., 1996). Biodegradation kinetics are also highly dependent on site characteristics (Dobbins et al., 1987) and vadose zone depth (Dobbins et al., 1987), but depth dependency can be highly irregular (Federle, 1988). Biodegradation of a spiked hydrocarbon into already-contaminated vadose materials may appear particularly slow if preexisting hydrocarbons are used as preferential substrates.

Chlorinated Solvents

Chlorinated solvents, which are heavier than water and tend to sink below the water table, can be important pollutants in the vadose zone where they partition into the vapor and solution phases and can exist as nonaqueous phase liquids. Reductive dehalogenation occurs un-

der anaerobic conditions that are not expected in the bulk vadose conditions. In a 1.5-m-long model vadose zone column with continuously flowing gas of He (carrier for perchloroethylene [PCE]), N_2, H_2 and CO_2, a consortia of pentachlorophenol-degrading bacteria effectively transformed vapor phase PCE (10 ppmv) methanogenically to trichloroethylene (TCE), dichloroethylene, and vinyl chloride (VC), the expected products of PCE reductive dechlorination in saturated systems (Mihopoulos et al., 2000). The reduction rates of PCE and its metabolites appeared to be first order and rapid compared with previous reports of aqueous phase kinetics (Mihopoulos et al., 2000). The main metabolite was VC (Mihopoulos et al., 2000), which is a suspected carcinogen (Alexander, 1999).

Vadose materials sampled at depth intervals up to 25 m from gasoline-contaminated sites and maintained at ambient moisture and nutrient content in the lab do not demonstrate either toluene (Fuller et al., 1995; Holden et al., 2001) or TCE biodegradation activity (Fuller et al., 1995). However, significant rates of TCE and toluene biodegradation occurred when inorganic nutrients were added, raising moisture contents to approximately 14% (Fuller et al., 1995). When slurried with nutrients, both TCE and toluene biodegraded slowly. Removal of TCE was incomplete (approximately 70 vs. 100% for toluene), and toluene addition was a prerequisite for TCE biodegradation (Fuller et al., 1995). In the slurried systems, rates of biodegradation of both TCE and toluene increased with sampling depth, and there was no lag phase observed in materials collected from either a gasoline-contaminated or uncontaminated site (Fuller et al., 1995). Addition of a pure culture of toluene-degrading bacteria isolated from the gasoline-contaminated vadose materials did not affect biodegradation of either TCE or toluene under slurried or unsaturated conditions (Fuller et al., 1995), suggesting that degradation rates were not limited by the population size of biodegraders. The requirement of toluene addition for TCE biodegradation (Fuller et al., 1995) suggests a cometabolic process with toluene as the primary C source. A similar phenomenon was observed with vadose material collected from the Savannah River Plant, where propane amendments increased TCE removal (Phelps et al., 1989). A lack of primary C sources in the deeper layers of the vadose zone could limit the in situ biotransformation of some chlorinated solvents by cometabolic bacteria. However, chlorinated solvents and water can leach soil OM from the vadose zone into groundwater, where this newly transported C can facilitate reductive dechlorination (Lyon et al., 1995).

Metals

Compared with organic pollutants, we know relatively little about the interactions between vadose zone microbes and metal pollutants. Vadose microbes can colonize stainless steel, nickel alloy, and titanium, but the extent of colonization is greatest at high relative humidity and moderate temperatures (Else et al., 2003). Microbially induced corrosion can occur across the range of environmental conditions found in the vadose zone (Hamilton, 1995), releasing metal into the soil solution. In general, the fate of solubilized metals in the vadose zone is strongly affected by microbes. For example, it appears that a wide variety of actively growing vadose zone microbes (i.e., gram-positive and -negative bacteria, and yeast) can prevent the sorption (effectively increasing the mobility) of soluble Cd^{2+} and Ni^{2+} to vadose zone tuffaceous earth (Brown et al., 1994). The mechanism, at least for decreased Ni^{2+} sorption, appeared to be a microbially mediated decrease in pH (Brown et al., 1994). The consequence in column studies was to enhance breakthrough (Brown et al., 1994), which translates into faster travel times in the vadose zone. The authors astutely point out that this would be problematic in a mixed waste bioremediation scenario because hydrogen ion generation concomitant with organic pollutant biodegradation could prevent the sequestration of metals (Brown et al., 1994). However, vadose zone microbes may also enhance the sorption of metals. For example, in laboratory isotherm experiments, a vadose zone isolate identified as a *Bacillus simplex* strain rapidly sorbed, in the order of affinity: Cd^{2+}, Ni^{2+}, Co^{2+}, and Sr^{2+} (Valentine et al., 1996). The sorptive capacity of this subsurface isolate was higher than for nonsubsurface derived isolates *B. subtilis* and *Escherichia coli* (Valentine et al., 1996). Interestingly it was *Bacillus megaterium* in the Brown et al. (1994) study that more significantly inhibited Ni^{2+} and Cd^{2+} sorption, indicating that species-level physiological differences tied to environmental conditions, rather than gross bacterial taxonomy, may be a more important determinant of vadose microbial roles in metal sorption.

In addition to effecting sorption, vadose zone microbes can enhance metal reduction, even under relatively well-aerated conditions. For example, in well-aerated batch reactors containing deep vadose sediments, only the addition of C (as molasses) and NO_3^- were required to stimulate the reduction (and subsequent immobilization) of Cr(VI) to Cr(III) (Oliver et al., 2003). This phenomenon has extremely important implications for the prevention of toxic Cr(VI) migration into groundwater underlying heavily contaminated sites.

COUPLED PHYSICAL AND BIOLOGICAL MODELING

Mathematical models can be used to understand the dynamics of nutrient or pollutant transport and biodegradation in the vadose zone (Pasteris et al., 2002). Ideally, such models can also be used to accurately predict the fates of nutrients or pollutants in the vadose zone environment. Coupled physical and biological models of a pollutant in the vadose zone need to account for the processes of diffusive or advective transport (whichever is dominant), sorption onto solids, partitioning into the gas phase, and biodegradation. Sorption and gas-phase partitioning are often assumed to be instantaneous and are entered as equilibria expressions. Biodegradation is assumed to occur in the aqueous phase only. Microbial biodegradation kinetics are often treated as

first-order processes (Bekins et al., 1998), which may be appropriate considering the limitations that diffusion can cause in porous media (Simoni et al., 2001). However, depending on the concentration of substrate, zero-order or mixed-order rate expressions may be more appropriate (Bekins et al., 1998). If significant microbial growth is assumed to occur during the process of biodegradation, the size of the microbial population must also be considered. The temperature dependency of microbial kinetics needs to be known or assumed. The depth-variant attributes of water content, nutrient concentration, temperature, and microbial population sizes through the vadose zone also need to be known or assumed.

There is no typical model to depict in this review. Rather, the reader is referred to a few references where coupled physical and biological models applicable to the vadose zone are reviewed (Criddle et al., 1991; Murphy and Ginn, 2000). For example, Pasteris et al. (2002) estimated first-order biodegradation rate constants using a coupled diffusion-reaction model for hydrocarbons released and monitored within in a field lysimeter (Table 5). Their reported first-order rate coefficients are for hydrocarbons in the gas phase; aqueous phase biodegradation rate constants would be corrected with water content data and Henry's constant (Pasteris et al., 2002). Another specific example applicable to the vadose zone is the development of a numerical simulation of soil vapor extraction and bioventing, which both involve advective air flow through the soil profile, called MISER (Rathfelder et al., 2000). In MISER, interphase mass transfer is modeled rather than making the assumption of instantaneous partitioning between phases. Also included are terms for microbial inhibition by pollutant substrates and kinetic parameters for metabolism of O_2 and another inorganic nutrient. MISER also accounts for reduced degradation during low O_2 as well as growth and decay of microbial populations (Rathfelder et al., 2000). The numerical simulations performed with MISER were highly sensitive to microbial kinetic parameters, suggesting that kinetic parameters should be accurately determined (Rathfelder et al., 2000).

In the models of Pasteris et al. (2002) and Rathfelder et al. (2000), the pollutant source was within and below the unsaturated zone, respectively. In a third example, Baek et al. (1989) simulated microbial biodegradation in the unsaturated zone when the substrates originate at the soil surface and percolate downward with water through the unsaturated zone. In their "downflow" scenario, the authors concluded that biodegradation below the soil surface is insignificant because of the low abundances of microbes in the deeper soil layers (Baek et al., 1989). However, they also admit that the microbial distribution with depth needs to be better characterized to accurately simulate biodegradation along the profile (Baek et al., 1989).

Why do so many estimates of biodegradation rates appear to follow first-order kinetics? One explanation is that data appear to fit a first-order function, but that it is the relative ease in model use that drives the choice of function when in fact a first-order relationship is not accurate (Bekins et al., 1998). When a first-order model is appropriate, an explanation is that the mass transfer of substrates, either at the local or microbe scale, is limiting microbial metabolism in the vadose zone environment. Harms (1996) clearly showed that, in unmixed saturated and unsaturated experimental systems, diffusion is the major factor limiting the microbial degradation of naphthalene. Hunt et al. (1995) predicted that diffusional limitations are at the scale of water films coating microbes in unsaturated soils, and that these diffusional limitations vary strongly with the solubility (in water and octanol) properties of various gas-phase hydrocarbons. Additionally, Holden et al. (Holden et al., 1997b) showed that biofilms cultivated in humid air were highly resistant to toluene diffusional mass transfer, and coefficients for diffusion in biofilm were 100 times less than for diffusion in water. These issues suggest that mass transfer may be the limiting factor in microbial metabolism in soils and that the mass transfer limitations, depending on the substrate, may be most manifest at the local or microbial scale.

Although the vadose zone is not a well-mixed system, it is sometimes modeled as a complete-mix reactor where microbes are not limited by diffusional or advective resupply of nutrients. In such cases, the concentration of growing microbes, X (m L^{-3}), increases according to a simple first-order (i.e., logarithmic) relationship with respect to time:

$$\frac{dX}{dt} = \mu X \qquad [2]$$

where μ is specific growth rate (t^{-1}).

Specific growth rate, for a particular microbe under a given set of environmental conditions, varies with substrate concentration, as per the Monod equation:

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad [3]$$

where μ_{max} is the maximum specific growth rate (t^{-1}), S is substrate concentration (mL^{-3}), and K_S = substrate concentration at one-half μ_{max} (mL^{-3}).

Equation [2] can be related to a change in substrate concentration with time by a yield coefficient, Y, where Y is mass of substrate utilized per mass of microbes. In that case, the rate of substrate depletion is

$$\frac{dS}{dt} = -\mu XY \qquad [4]$$

An equation that is analogous to Eq. [3] but more applicable to substrate depletion by nongrowing microbes is the Michaelis–Menten equation:

$$\nu = \frac{\nu_{max} S}{K_m + S} \qquad [5]$$

where ν is substrate depletion rate ($mL^{-3}\ t^{-1}$), ν_{max} is maximum substrate depletion rate ($mL^{-3}\ t^{-1}$), S is substrate concentration (mL^{-3}), and K_m is substrate concentration at one-half ν_{max} (mL^{-3}).

Note that Eq. [5] describes the variable ν whose units are different from the Monod specific growth rate, μ, because ν describes the mass of substrate depleted per

unit volume of catalysts (free enzymes or nongrowing microbes).

As mentioned above, transport processes may frequently limit the observed rate of microbial reaction in the vadose zone. At the microbe-scale, the EPS that commonly surrounds cells can significantly impede the diffusion of nutrients to biofilm bacteria. If we assume steady-state conditions across the biofilm–microbe boundary, the applicable differential equation that accounts for both diffusional substrate resupply and microbial reaction is

$$D\frac{d^2S}{dy^2} = \mu XY\,\eta_i \qquad [6]$$

where D is the effective diffusivity of limiting nutrient ($L^2\,t^{-1}$), y is the distance along the axis of diffusion (L), and η_i is internal effectiveness factor, a dimensionless parameter that reduces microbial reaction to the rate constrained by diffusional rate limitation

The boundary conditions for Eq. [6] are: for $y = 0$, S = a maximum concentration occurring at the outside edge of the biofilm; for $y = L$, $dS/dy = 0$.

Equation [6] can be used to describe gas phase substrate mass transfer and microbially mediated substrate reactions along a depth profile, as long as the advection of gas through soil pores is relatively rapid and there are no rate-limiting sorption processes. Where mass flow through soil pores and/or sorption processes limit the rate of biogeochemical processes in the vadose zone, the mathematical model must include explicit mass flow terms for the moving phase. Equations for describing mass flow of solution phase nutrients or pollutants are typically based on Darcy's equation as described elsewhere (e.g., Freeze and Cherry, 1979; Hillel, 1980; Jury et al., 1991).

CONCLUSIONS

Although the vadose zone is physically, chemically, and microbiologically heterogeneous (Bone and Balkwill, 1988), some generalizations can be made regarding microbes and microbial processes in the vadose zone. The presence of microbes throughout the vadose zone has been well documented. The abundance of bacteria decreases with vadose zone depth, largely due to the decrease in organic C concentrations with depth. Preferential flow paths and macropores are localized sites of high nutrient availability that contribute to the observed spatial heterogeneity in microbial abundances and processes in the vadose zone. The physicochemical properties of pollutants alters their accessibility to microbes residing in the vadose zone and the kinetics of the pollutant biodegradation. Thus, while microbial respiration of simple substrates declines with depth as population sizes decline, these patterns of activity are not necessarily good predictors of biodegradation patterns for pesticides and other pollutants.

The vadose zone is often treated as a relatively static and segregated entity, with gradients from top to bottom of nutrients, temperature, and microbes. However, the capillary fringe is also an important component of the vadose zone. What distinguishes the capillary fringe from regions above and below are the transience of the water table and thus frequent rearrangement of gas, liquid (aqueous and non-aqueous), and solid (biotic and abiotic components). Such rearrangements may increase contact between catalysts and substrate and thus make for a rich catalytic region. Microbial communities residing within the capillary fringe are likely to be distinct from the communities above and below since they would be selected to cope with shifts in oxidation–reduction potentials and nutrient availability.

Coupled biological and physical models are highly sensitive to assumptions regarding microbial interactions (e.g., predation or competition) and microbial kinetics (Travis and Rosenberg, 1997), yet the ecology of vadose zone bacterial processes, including biodegradation, is not well understood. Several studies and mathematical models suggest that mass transfer may be an important factor controlling pollutant biodegradation in the vadose zone. We do not know how mass transfer limitations are manifested at different spatial scales and how microbial growth habits influence diffusional processes across a range of vadose zone types (Dohse and Lion, 1994; Fuller and Scow, 1997).

Microscale phenomena seem to have an important influence on microbial life in the vadose zone. What governs the spatial orientation or patchiness of surface colonization in the vadose zone? Even in surface soils, only 0.17% of organic and 0.02% of sand surfaces are colonized (Hissett and Gray, 1976). In surface soils, the highest density of bacteria is by far on organic particles (Hissett and Gray, 1976). Perhaps this spatial association of bacteria with C in surface soils occurs in subsurface soils and accounts for the strong correlation between the two variables with depth. Yet, much of the C in deeper soils has resided there for a very long time, suggesting that if bacteria are colonizing organomineral complexes at depth, then the quality of that C or other nutrient limitations must prevent rapid C mineralization. Therefore, an important gap in knowledge exists regarding the spatial and temporal organization of microbes in the vadose zone, the relationship with nutrient sites, the architecture of microbial habitats, and how these factors influence microbial processes. More importantly, how can knowledge of microsite phenomena be used to build accurate and predictive models of vadose zone microbial processes?

Lastly, there are a host of fundamental issues in microbial ecology that may be particularly relevant to understanding microbial processes in the vadose zone. Such issues may include quorum sensing between bacteria, the range of electron acceptors available and used, interactions between populations and community assemblages, and the transfer of genetic material between distinct phylogenetic groups of microbes. There needs to be a better understanding of these and many other issues in vadose zone microbial ecology, particularly given the importance of vadose zone microbes in attenuation of nutrients and pollutants in the subsurface.

ACKNOWLEDGMENTS

We acknowledge funding from the NSF Microbial Observatories Program, an NSF Microbial Biology Postdoctoral Fel-

lowship to NF, by the UCSB-LANL CARE program, and technical assistance from Eloise Marszalek.

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