Consider the Anoxic Microsite: Acknowledging and Appreciating Spatiotemporal Redox Heterogeneity in Soils and Sediments

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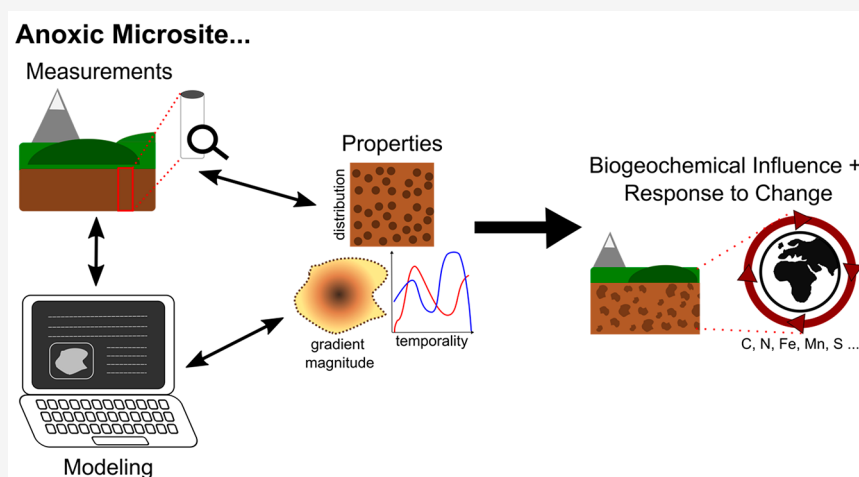
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ABSTRACT: Reduction−oxidation (redox) reactions underlie essentially all biogeochemical cycles. Like most soil properties and processes, redox is spatiotemporally heterogeneous. However, unlike other soil features, redox heterogeneity has yet to be incorporated into mainstream conceptualizations of soil biogeochemistry. Anoxic microsites, the defining feature of redox heterogeneity in bulk oxic soils and sediments, are zones of oxygen depletion in otherwise oxic environments. In this review, we suggest that anoxic microsites represent a critical component of soil function and that appreciating anoxic microsites promises to advance our understanding of soil and sediment biogeochemistry. In sections 1 and 2, we define anoxic microsites and highlight their dynamic properties, specifically anoxic microsite distribution, redox gradient magnitude, and temporality. In section 3, we describe the influence of anoxic microsites on several key elemental cycles, organic carbon, nitrogen, iron, manganese, and sulfur. In section 4, we evaluate methods for identifying and characterizing anoxic microsites, and in section 5, we highlight past and current approaches to modeling anoxic microsites. Finally, in section 6, we suggest steps for incorporating anoxic microsites and redox heterogeneities more broadly into our understanding of soils and sediments.

KEYWORDS: *anoxic microsite, oxygen, soil, biogeochemistry, redox heterogeneity*

1. INTRODUCTION

Reduction−oxidation (redox) reactions, the biotic or abiotic transfer of electrons, underlie essentially all biogeochemical cycles. Redox constrains the energetic bounds of cellular growth, governs the bioavailability of nutrients and contaminants, and drives chemical weathering of rocks and minerals. In soils, redox reactions are paramount as they connect elemental cycles across the atmosphere, biosphere, hydrosphere, and lithosphere, the mixed media of soils.

Oxygen (O_2) is a central regulator of soil redox cycling. A potent oxidant, O_2 facilitates abiotic redox transformations, particularly at redox interfaces, where strong oxidizers (e.g.,

O_2) and reduced species meet. Oxygen also has a pronounced effect on microbial respiration, the principal biotic driver of soil redox cycling. Respiration represents an essential series of redox reactions for most organisms, providing energy through electron movement from one chemical species (i.e., the

electron donor) to another (i.e., the electron acceptor). Oxygen is the most energetically favorable electron acceptor. Thus, when O_2 is present (i.e., oxic conditions), O_2 is primarily utilized as the terminal electron acceptor and aerobic respiration proceeds. However, when O_2 declines below a physiological threshold (i.e., anoxic conditions), microorganisms can utilize alternative electron acceptors and perform anaerobic respiration or fermentation. Anaerobic respiration yields considerably less energy than aerobic respiration, resulting in slower substrate consumption and organism growth.[1]

Soil O_2, and thus redox status, is heterogeneous across both space and time. Redox heterogeneity primarily has been defined at the landscape scale, vis-à-vis wetland and aquifer delineation, with some attention granted to soils undergoing redox fluctuations over time.[2] Generally, flooded soils, such as those in lowland areas, are assumed to be anoxic,[3] while upland soils are assumed to be entirely oxic and, thus, dominated by oxygen-dependent redox processes. However, redox heterogeneity can also occur at finer scales, within bulk oxic soils and over shorter periods of time. Seemingly aberrant biogeochemical behavior is often attributed to redox heterogeneity in soils,[4−10] yet there are few studies that directly investigate the influence of redox heterogeneity on biogeochemistry.

Redox heterogeneity is not unique: nearly all soil properties and processes are heterogeneous across space and time. However, unlike redox, other aspects of soil heterogeneity are better defined. Small-scale and short-term spatiotemporal variability in soil biological activity is represented by biological "hot spots" and "hot moments",[11] whereas larger scale and longer term variability can be classified by biome and seasonal effect.[12−14] Soil structural units (e.g., aggregates) are a defining feature of soil pore heterogeneity,[15−18] and soil textural classes arose from an attempt to classify heterogeneity in the soil particle size distribution across landscapes.[19] At landscape scales, rainfall patterns, macrotopography, and groundwater intrusion dictate when and where soils receive and accumulate moisture,[19] and at smaller scales, macropores and preferential flow paths guide water and associated resources to some portions of soil while leaving other portions without.[20]

Defining the features of soil heterogeneity and clarifying the relationships between these heterogeneities across scales has advanced our understanding of soils as a complex system. Biological activity in soils depends on water, and thus preferential flow, for microbial habitat space and substrates for metabolism.[21−25] Preferential flow is dictated by soil structure,[20] and in turn, soil structure and preferential flow can be driven by texture and biological activity.[17,26,27] Environmental forcings (e.g., precipitation, snow melt, temperature) modulate these relationships over days, months, and years. For instance, freeze−thaw cycles alter soil pore size and connectivity as well as water and nutrient availability, which subsequently control soil microbial activity.[28−30] Alternatively, temperature-driven changes in root architecture can alter the distribution of soil root channels,[31] which serve as both effective conduits for water[22,32,33] and specialized habitats for microorganisms.[11,34] Clarifying how redox heterogeneity emerges from other physical and biological soil heterogeneities promises to advance our understanding of soil biogeochemistry.

Anoxic microsites, nonmajority soil pore volumes that are without O_2, are a defining feature of soil redox heterogeneity in bulk oxic soils and sediments. In the early 1960s, Currie

(1961) postulated that based on the laws of gaseous diffusion, soil aggregates may be anoxic at their cores.[15] Years later, microelectrode measurements confirmed localized anoxia at the interior of both saturated[35] and unsaturated[36] aggregates. Evidence of anoxic microsites has been observed in soils across multiple ecosystems, such as tropical and temperate forests, agricultural lands, deserts, and floodplains.[37−41] Increasingly, anoxic microsites are recognized as an important but ill-defined control on soil greenhouse gas emissions[42−44] and contaminant transport.[45,46] Yet, despite their prevalence, long-known presence, and presumed importance, our understanding of anoxic microsites remains incomplete. The soil properties and processes that shape anoxic microsite dynamics have only been partially characterized, and the influence of anoxic microsites on various biogeochemical cycles is still undefined.

In this review, we propose that anoxic microsites are a critical component of soil function and that failure to acknowledge soil redox heterogeneity limits our understanding of soil and sediment biology, mineral transformations, nutrient and contaminant dynamics, and biogeochemical cycling more broadly. In section 2, we define anoxic microsites and their mutable properties, specifically anoxic microsite distribution, redox gradient magnitude, and temporality. In section 3, we describe how anoxic microsites may alter cycles of key redox-active elements. In section 4, we review and evaluate methods for identifying and characterizing anoxic microsites, and in section 5, we review existing models that represent anoxic microsites and their environmental significance. Finally, in section 6, we suggest steps to incorporate anoxic microsites, their measure, and influence into the canons of soil biogeochemistry.

2. DEFINING ANOXIC MICROSITES

2.1. Formation. Anoxic conditions establish when O_2 supply is slower than O_2 demand. Oxygen supply in soils is controlled predominantly by water content and soil structure. Oxygen diffuses 10 000 times more slowly through water-filled pores than through air-filled pores,[15] and as a result, most submerged soils are considered bulk anoxic.[3] Even in seemingly well-drained forest soils, both long- and short-term precipitation have been associated with O_2 depletion[47] and redox heterogeneity.[48] Soil structure and texture also control soil O_2 supply. Clay particles flocculate and, with organic matter, aggregate. The continued expansion/contraction of aggregates—through hydration and thermal cycles and/or the action of plants roots and soil organisms—results in the formation of soil structural units, which can range from microaggregates, tens of micrometers in diameter, to peds, a few centimeters in diameter.[18,19] The small, tortuous pores characteristic of these units retain water across a wide range of soil moistures and extend the diffusion path length of O_2, impeding O_2 supply.[15,36,49−51]

Oxygen demand in soils is controlled principally by microbial demand for O_2 during aerobic respiration, though abiotic reactions may also deplete soil O_2.[10,52] The rate and magnitude of microbial O_2 demand varies with resource availability and temperature.[53−56] For example, electron-donor availability, most notably organic carbon (OC), partially dictates soil O_2 content. Higher concentrations of bioavailable OC are frequently linked to decreased O_2 concentrations as a result of enhanced aerobic respiration.[51,57] Soil moisture, in addition to inhibiting O_2 supply, can stimulate respiration and increase soil O_2 demand by increasing the connectivity of soil
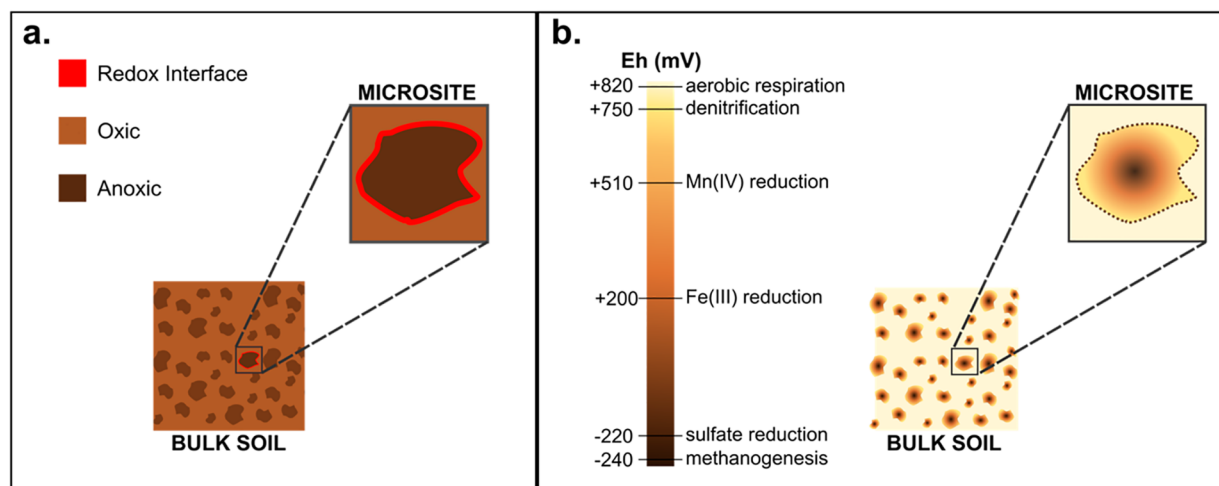
Figure 1. Depictions of anoxic microsites: (a) basic conceptualization of anoxic microsites, highlighting the redox interface, the boundary where oxidants and reductants meet; (b) anoxic microsites as redox gradients, hosting a multitude of microbial metabolisms.
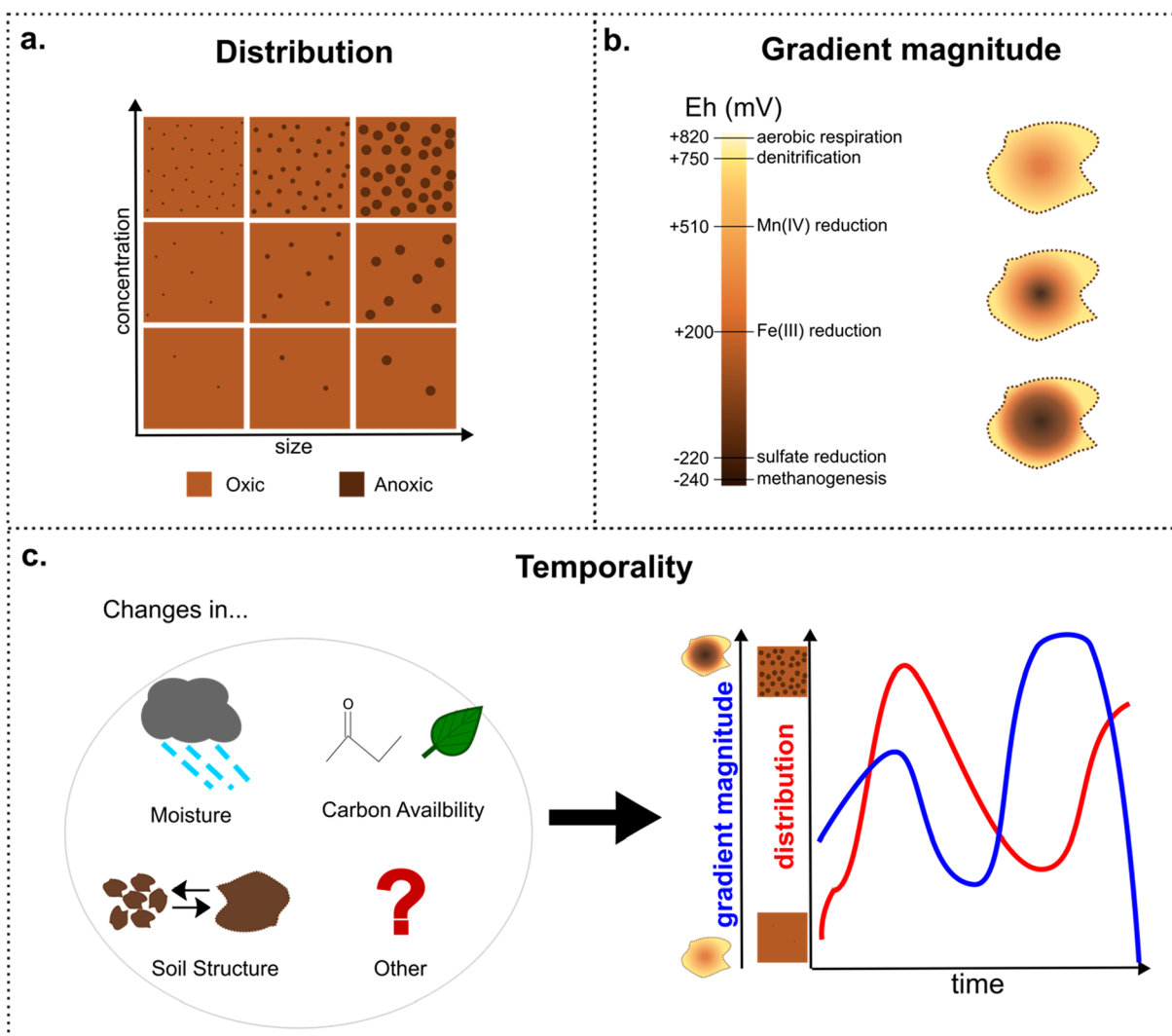

Figure 2. Representations of anoxic microsites: (a) distribution, (b) gradient magnitude, and (c) temporality.

pores, allowing mobile, dissolved OC to be transported to decomposer communities.[58] Temperature can also alter microbial activity, with decomposition rates and O_2 con-

sumption accelerating in warmer temperatures[59−61] and slowing in cooler temperatures.[56]

Anoxic microsites are emergent features of heterogeneous O_2 supply and demand within bulk oxic soils. Thus, anoxic microsites form in soil volumes with limited O_2 supply, excessive O_2 demand, or a mild imbalance of the two. The interior of aggregates or peds, common locations for anoxic microsites,[15,36,49] represents zones of limited O_2 supply. Anoxic microsites have also been associated with actively growing plant roots[62] and preferential flow paths,[24] both of which represent zones with ample bioavailable OC and, therefore, high O_2 demand. Anoxic microsites are also frequently observed within or near plant detritus;[40,63,64] these microsites are attributed to both high biological O_2 demand, as microorganisms break down and respire OC, and impeded O_2 supply, as plant tissues readily absorb water.[63]

2.2. Expanding and Revising Terminology.
The phrase "anoxic microsite" is imperfect. The prefix "micro-" connotes small spatial scales, yet anoxic microsites represent sizes ranging from micrometers to decimeters;[36,51,65] the definition of microsite is entirely dependent on one's frame of reference. The term "anoxic" emphasizes O_2 presence and absence (Figure 1a), yet the biogeochemical influence of anoxic microsites is more nuanced. Anoxic microsites represent gradients in redox potential below the anoxic threshold and may host a multitude of chemical transformations at various distances from each other (Figure 1b). Additionally, the exterior of an anoxic microsite represents a critical redox interface where reduced species and strong oxidants meet (Figure 1a). Finally, the suffix "-site" implies a false sense of constancy. The word "site" (e.g., building site, camp site, archeological site) often refers to exact locations of structures that last for days or years, whereas the lifetime, size, and location of an anoxic microsite is likely shorter, variable, and still largely unknown.

Despite its imperfections, the term anoxic microsite has merit. The term anoxic centers the importance of O_2 in regulating biogeochemical cycles, providing a single metric by which we may define a region of interest. The term microsite provides some sense of relative scale: in a soil containing anoxic microsites, the anoxic portion of the sediment represents the minority of soil pore space. Beyond anoxia and minority soil volume, anoxic microsites can exhibit a variety of characteristics. To examine the diversity that exists within the anoxic microsite classification and highlight the biogeochemical influence of anoxic microsites, we define three domains of variability in how anoxic microsites manifest: anoxic microsite distribution, redox gradient magnitude, and temporality (Figure 2).

Anoxic microsite distribution, the size and location of anoxic microsites, ultimately determines the susceptibility of anoxic microsites to disturbance and the absolute volume over which anaerobic processes may occur. Anoxic microsite distribution theoretically ranges from small and sparse (Figure 2a, lower left) to large and highly concentrated (Figure 2a, upper right). Small and sparse distributions result in less anoxic volume over a given space than large and concentrated anoxic microsites. Additionally, anoxic microsites can vary in size within the same soil profile[36] and can be unevenly distributed, clustering in specific horizons or near plant roots and preferential flow paths.[24,62,66]

Redox gradients within anoxic microsites represent vector quantities, both a direction and a magnitude of change. Often, anoxic microsites are depicted as centered on areas of limited diffusion, such as aggregates, containing a singular redox minimum toward the center of the aggregate (Figure 1b). In reality, an anoxic microsite may contain multiple redox minima, asymmetrical redox gradients,[36] or redox minima centered on areas of preferential flow, such as the exterior of an aggregate.[24,57] The orientation of redox minima within a single anoxic microsite likely alters microsite biogeochemical function. We limit our discussion to the influence of redox gradient magnitude as the principals of redox gradient magnitude can be applied across multiple microsite orientations and scales.

Redox gradient magnitude governs (i) the extent of reducing conditions in anoxic microsites and (ii) the distance reduced and oxidized species must diffuse to react with other oxidizing or reducing species, respectively (examples provided throughout section 3). As a result, redox gradient magnitude controls the processes that occur within and near anoxic microsites. In our usage, large redox decline over a confined distance constitutes a sharp or steep gradient (Figure 2b, lower panel), whereas a smaller redox decline over the same distance constitutes a milder gradient (Figure 2b, upper panel). Gradient magnitude within anoxic microsites depends on the supply of electron donors (e.g., OC) and acceptors, which is constrained by donor/acceptor concentration and their ability to move through soil pore space (i.e., diffusion).[67] For example, supplying nitrate (NO_3^-; i.e., an electron acceptor) to an anoxic microsite that supports Fe reduction effectively decreases redox gradient magnitude. Given sufficient time and limiting OC or nutrients, providing an alternative, more favorable electron acceptor to pair with the oxidation of OC can shift the dominant respiration pathway(s) of a system.[68] Alternatively, supplying excess OC increases redox gradient magnitude by increasing demand for and depletion of electron acceptors over a confined distance.[69] Due to their influence on diffusive flux, soil structure and texture may attenuate or amplify the response of gradient magnitude to changes in electron-donor and -acceptor availability.[24,67]

Anoxic microsite temporality or transience represents the variable frequencies with which anoxic microsites are present and the fluctuations of redox gradient magnitude. The temporality of anoxic microsites determines the net flux of a given element over a constrained period. Anoxic microsites can endure for hours or months[24,51,70] and possibly from seconds to years. Anoxic microsite temporality is driven by changes in soil O_2 supply and demand that result from changes in moisture, OC availability, soil structure, and potentially other drivers (Figure 2c). Climatic (e.g., precipitation, wet−dry, freeze−thaw), biotic (e.g., plant root exudates, earthworm burrows), and anthropogenic (e.g., farming practices like tillage, OM amendments) perturbations and dynamics drive changes in soil moisture, OC availability, and structure and, in turn, likely alter anoxic microsite temporality.[17,24,71,72]

3. INFLUENCE OF ANOXIC MICROSITES ON BIOGEOCHEMICAL CYCLES

Classic soil dogma asserts that upland soils and bulk oxic aquifers exclusively host aerobic biogeochemical transformations.[73] However, there is increasing evidence that anoxic microsites exist within bulk oxic soils and sediments, dictating the fate of several elements; the precise influence of anoxic microsites on various biogeochemical cycles is still being investigated. Here, we explore the various redox processes that may be hosted within and near anoxic microsites using anoxic microsite distribution, gradient magnitude, and temporality to

discuss how different manifestations of anoxic microsites can have varying effects on key redox transformations. Where possible, we cite anoxic microsite-specific literature. Otherwise, we apply knowledge about anaerobic processes to hypothesize how anoxic microsite presence and variability may alter elemental cycles.

3.1. Soil Organic Carbon.

Anoxic microsite distribution can alter the fluxes and distribution of soil OC, but evidence is mixed as to whether anoxic microsites prompt soil OC accrual (i.e., decreased oxidation of OC to CO_2) or loss (i.e., enhanced oxidation of OC to CO_2). Anoxic conditions can slow the oxidation of OC to CO_2 by (i) precluding the oxidative depolymerization of large macromolecules[68,74,75] and (ii) decreasing the energetic yield of microbial respiration.[1,76,77] Thus, anoxic microsites and proxies for anoxic microsite volume, such as soil moisture or $Fe(II)$ concentration, are often positively correlated with soil OC content in bulk oxic soils.[78,79] Similarly, soils that support more anaerobic respiration, as indicated by $Fe(II)$ and methanogen abundance, have been shown to lose the most OC upon oxygenation,[72] and prolonged O_2 limitations within soil structural units can partially explain discrete zones of relatively old OC.[66] However, anoxic conditions can also prompt the reductive dissolution of Fe−oxides (see section 3.3), which can liberate mineral-bound OC, increasing microbial access to OC.[80] Under unsaturated, intermediate moisture contents (~75% saturated), Huang and Hall (2017) attributed elevated soil OC loss as CO_2 and CH_4 to anoxic microsites and the reductive dissolution of Fe−oxides.[81] Yet, the balance between OC accrual and loss within anoxic microsites and subsequent impacts on ecosystem-scale OC cycling remain undefined.

Redox gradient magnitude determines the rate of soil OC loss and/or accrual in anoxic microsites as well as the identity of the OC byproducts. Naughton et al. (2021) decreased redox gradient magnitude in model anoxic microsites by adding NO_3^-, a relatively favorable electron acceptor.[68] The decrease in gradient magnitude increased respiration (i.e., OC oxidation) by enabling denitrification, which proceeds more quickly than OC oxidation paired with sulfate (SO_4^{2-}) or Fe reduction. Redox gradient magnitude also determines whether anoxic microsites can support methanogenesis. Methanogenesis is one of the least thermodynamically favorable respiration pathways. Thus, methanogens are outcompeted for OC and nutrient resources when other electron acceptors and hence more energetically favorable respiration pathways are present.[3] However, in the absence of OC limitations, methanogenesis can proceed alongside other anaerobic respiration pathways.[82] Evidence of methanogenesis has been observed in bulk oxic tropical, desert, arid agricultural, Mediterranean grassland, and temperate forest soils,[37,38,72,83−85] indicating that these soils host anoxic microsites with redox declines large enough to support methanogenesis. Similarly, redox gradient magnitude determines the fate of specific OC compounds. Soil OC with a nominal oxidation state of C of less than −1.8 or −0.8 is thermodynamically nonrespirable when paired with the reduction of $Fe(III)$ or SO_4^{2-}, respectively.[43] As a result, reduced (low nominal oxidation state of C) OC compounds, such as lipids, accumulate within sulfidic and/or iron-reducing anoxic microsites[39,67] but not within denitrifying microsites.

Environmental forces, such as weather events and climate, drive anoxic microsite temporality, influencing net C fluxes over time. In humid tropical soils, dry periods of more than 2 days were associated with concomitant declines in methane (CH_4) emissions, presumably due to the aeration of anoxic microsites.[71] High-latitude upland soils typically serve as net CH_4 sinks, but during the nongrowing season, soils can become a net source of CH_4 depending on soil moisture and temperature.[86] Similarly, precipitation events and generally rainy seasons can support anaerobic respiration of OC not observed in drier periods[10] or turn upland temperate forest soils into temporary CH_4 sources.[4] In laboratory systems, relative enrichment of a low nominal oxidation state of C compounds was established in less than 40 days,[67,68] and net CH_4 production from a bulk oxic biological soil crust was observed in less than 7 days.[84] However, the intensity of environmental forcings and minimum duration over which microsites must persist to induce observable changes in C flux or speciation remains unknown.

3.2. Nitrogen.

Anoxic microsite distribution partially determines N retention and loss from ecosystems. Anoxic soils favor microbial denitrification, the reduction of NO_3^- to dinitrogen gas (N_2),[87] with intermediates nitric oxide (NO) and nitrous oxide (N_2O), potent greenhouse gases that often escape full metabolization and contribute to global warming.[88] Denitrification is seemingly ubiquitous across upland soils and is regularly observed in agricultural fields, peatlands, and humid tropical forests.[36,89,90] Net denitrification and N loss from soils is associated with anoxic microsite abundance, with variance in denitrification potential over larger (i.e., meter) scales attributed to key drivers of anoxic microsite formation, such as high water and organic matter contents.[91−93] However, denitrification is dependent in part on adequate OC and NO_3^- supply.[94,95] As a result, denitrification scales nonlinearly with anoxic volume.[36,96] The relative positioning of anoxic microsites alters denitrification rates and the proportion of denitrification byproducts. Schlüter et al. (2019) embedded model aggregates in layered and random orientations and found that more evenly spaced anoxic microsites (i.e., random orientation) resulted in greater denitrification rates as well as increased NO and N_2O product ratios.[97] Finally, the absolute location of anoxic microsites can dictate N availability. For example, anoxic microsites associated with plant roots may limit plant N availability by hosting redox interfaces that promote rapid denitrification and/or nitrification, resulting in gaseous or aqueous N loss.[98−100]

The relationship between microsite redox gradient magnitude and N cycling is complex. Gradient magnitude controls N response to anoxic microsites. However, influxes of N, particularly NO_3^-, can alter redox gradient magnitude, with enhanced NO_3^- supply decreasing redox gradient magnitude.[68] At the exterior of an anoxic microsite, coupled denitrification−nitrification results in constant cycling between nitrite (NO_2^-) and NO_3^-,[89,101−103] with nitrification supplying the NO_3^- precursors for denitrification and possibly enhancing N_2O production.[102] Microbial anammox functions similarly, with NO_2^- or NO_3^- supplied at the oxic−anoxic interface fueling anaerobic oxidation of ammonium (NH_4^+) to N_2 or the intermediate byproduct, N_2O.[104−106] In addition to being oxidized at the oxic−anoxic interface, NO_2^- can act as an oxidant when reacting with other anaerobic byproducts. During chemodenitrification, NO_2^- oxidizes $Fe(II)$ to produce $Fe(III)$ and N_2O.[107,108] During nitrite dependent anaerobic methane oxidation (n-DAMO), NO_2^- oxidizes CH_4 to produce CO_2 and N_2, thereby mitigating CH_4 and N_2O emissions.[105,109−112] Chemodenitrification has been directly

observed in anoxic microsites,[113] but n-DAMO has only been observed in flooded paddy soils thus far.[114] Gradient sharpness presumably controls the likelihood that anoxic microsites will host chemodenitrification or n-DAMO. If the zone of Fe reduction within the gradient is relatively close to the nitrite byproduct, chemodenitrification may be more likely to occur. Conversely, if the zone of methanogenesis is physically separated from a suitable NO_2^- supply, n-DAMO may be less likely to occur.

Anoxic microsite temporality drives pulses in N loss from soils. Once anoxic or hypoxic (low-oxygen) conditions are established, denitrifiers respond quickly due to their often facultative nature, high abundance (up to 20% of culturable taxa),[115] and rapid production of denitrifying enzymes.[116,117] As a result, even ephemeral anoxic microsites caused by heavy rains, soil freeze−thaw, or even drought-induced cell lysis can result in significant N_2O emissions.[113,118,119] Sustained patterns in O_2 availability, such as fluctuating or static redox conditions, can also alter the identity of N byproducts from soils. For example, Pett-Ridge et al. (2013) found that soils under fluctuating redox conditions supported more nitrification than soils under static oxic or anoxic incubations.[120] Static anoxic conditions are more likely to facilitate complete denitrification, reducing oxidized N species fully to N_2[121] and limiting greenhouse gas production. Similarly, static anoxic conditions are more likely to promote dissimilatory nitrate reduction to ammonium,[87] limiting aqueous N loss.

3.3. Iron and Manganese. Anoxic microsite distribution dictates the volume and precise location of Fe and Mn redox transformations and thereby affects soil development, nutrient acquisition, and contaminant mobilization. Although the reduction of Mn−oxides is thermodynamically more favorable than the reduction of Fe−oxides,[3] Fe is more abundant and thus often accounts for the majority of soil anaerobic respiration.[67] Sustained or repeated Fe reduction within anoxic microsites can result in profile- and feature-scale Fe loss as Fe(II). For example, along a Hawaiian precipitation gradient, wetter bulk soils have lower Fe−oxide and oxyhydroxide contents than drier bulk soils.[122] Similarly, anoxic microsites formed within and near root channels drive Fe reduction and depletion over pedogenic time scales.[123] Reductive dissolution of Fe−oxides and Mn−oxides mobilizes OC,[81,124,125] P,[126,127] nutrient cations,[128] and contaminants[129] bound to these Fe−oxides and Mn−oxides. For example, in agricultural soils, anoxic microsites in a compacted plough layer drove reduction of Fe−oxides and Mn−oxides and comobilized P into soil water.[130] Similarly, rhizosphere-associated anoxic microsites have been shown to enhance ferrihydrite dissolution and mobilize adsorbed As and U, facilitating plant uptake of these contaminants.[131]

Redox gradients within anoxic microsites control the colocation of Fe, Mn, and other reactants, thereby controlling which Fe and Mn transformations occur within or near anoxic microsites. Several Fe and Mn redox reactions are facilitated at redox interfaces.[132,133] Microbial Fe oxidizers are often situated in microaerophilic zones at oxic−anoxic interfaces and conserve energy by oxidizing Fe(II) to Fe(III).[134,135] Fe(II) can also be oxidized to Fe(III) by abiotic reaction with molecular O_2 or hydrogen peroxide (i.e., the Fenton reaction), the byproducts of which include Fe(III) and highly reactive hydroxyl radicals.[136,137] Similarly, Mn(II) can be oxidized abiotically to Mn(III/IV) by reaction with reactive oxygen species[138] or O_2 given sufficiently high pH.[137,139] Sharp redox

gradients shorten the diffusion path length between Fe(II), Mn(II), and the oxic interface, increasing the likelihood of the Fenton reaction and/or Mn oxidation.[132,136] As a result, anoxic microsites with sharper redox gradients may produce more reactive oxygen species and Mn(III) at their exterior than microsites with milder gradients. Gradient magnitude also controls Fe and Mn transformations strictly within the anoxic microsite. For example, Pallud et al. (2010) imposed a sharp redox gradient within a model aggregate system by increasing the concentration of electron donors.[69] Concentrations of Fe(II) and inorganic carbon were high enough to promote siderite ($FeCO_3$) formation, whereas in a shallower gradient system, no siderite was formed. Similarly, gradient sharpness partially controls the likelihood of chemodenitrification, the abiotic oxidation of Fe(II) by NO_2^- (see section 3.2).[140]

Finally, anoxic microsite temporality controls the net fluxes and byproducts of Fe and Mn throughout soils. Frequent transitions from anoxic to oxic conditions can increase Fe−oxide crystallinity, decreasing the surface area, sorbent capacity, and reducibility of these oxides.[122,141−143] If the same volume of soil repeatedly endures redox fluctuations, distinct patches of soil, such as the rhizosphere, may contain more crystalline Fe−oxides over pedogenic time.[144] Changes in anoxic microsite distribution and gradient magnitude are often driven by changes in seasons, influencing the mobility of Fe and Mn from soils. For instance, warm, wet conditions in summer months may be more likely to support anoxic microsite formation and persistence, leading to greater soluble Fe, Mn, and P in summer months than in cool, dry winter months.[130]

3.4. Sulfur. Anoxic microsite distribution partially dictates the mobility of S within soils and sediments. Within anoxic microsites, microorganisms use SO_4^{2-} as an electron acceptor, producing aqueous sulfide (HS^-/S^{2-}) and/or hydrogen sulfide (H_2S).[145] These sulfide species can react with transition metals to form low-solubility elemental S and/or (di)sulfide minerals, essentially immobilizing S,[145−148] contaminant metals, and OC from soil and groundwater.[149−161] Thus, anoxic microsites can export or immobilize S, with S mobility depending on the geochemistry of the anoxic microsite. For example, immobilization of sulfide via reductive dissolution of Fe(III)−(hydroxy)oxides and Fe−sulfide formation requires high ratios of S(II) to Fe(II).[162−164] However, in systems with inadequate S^{2-} or excess Fe(II), anoxic microsites mobilize S and associated nutrients and contaminants as colloids.[165] The export of these sulfidic colloidal species can extend the boundaries of anoxic microsites by stimulating microbial activity and accumulation of reduced S products downgradient of the "original" anoxic microsite.[166,167]

Redox gradient magnitude also determines the fate of S in sediments with anoxic microsites. Sulfate reduction yields less energy to microorganisms than other electron acceptors, and therefore, SO_4^{2-} reduction is often limited or inhibited by other electron acceptors of higher redox potential.[3,168] Thus, anoxic microsites with mild redox gradients are less likely to support SO_4^{2-} reduction and S^{2-} generation than similarly sized anoxic microsites with sharper redox gradients. Gradient magnitude also dictates the distance S^{2-} species must travel to encounter oxidizing conditions. For example, hydrogen sulfide can react with Fe(III)−oxides to produce Fe(II) and elemental S.[169] However, the likelihood of this interaction is dependent on gradient sharpness, with sharper gradients (i.e., shorter distances between sulfidic species and Fe(III) species)

Table 1. Techniques for Identifying and Characterizing Anoxic Microsites

approach	scale(s)	advantages	disadvantages
Bulk and 1D			
oxygen probes	>5 μm	direct measurement of anoxic/oxic conditions in situ deployment	false negatives; limited information on how much Eh declines below anoxic threshold
Eh probes	from 5 μm to multimillimeter	in situ deployment reveals possible terminal electron acceptors	difficult to link to specific redox transformations
mediated amperometry	~100 mg of soil	provides reasonable averages for electron-accepting/donating capacity over small volumes	cannot implement in situ
redox-active species	see below	reveals specific biogeochemical transformations	false negatives
gaseous	from 1 g of soil to field	real-time indicator for anoxic microsites	consumption/production dynamics obscure data
solid/dissolved	~0.25–0.5 g of soil	relatively easy to measure	may not reflect conditions at time of sampling
microbial	~0.25–5g of soil	indicator in real time and/or for the recent past	limited spatial resolution
16S		fairly common technique; enables use of "big data"	phylogeny does not reveal function
SIP/BONCAT		links phylogenetics to function	technically cumbersome
qPCR/ddPCR		reflect microbial function and/or metabolic capacity	difficulties with environmental samples
2D			
planar optodes:	<1 mm	high resolution	unavoidable "wall" effect
O_2		optodes are reusable	difficult to implement in field
H_2S or Fe		optode reactivity is irreversible	optodes are single use (i.e., irreversible)
CARD-FISH	~0.5 cm^3 of soil	links microorganisms to microstructural features	thin sectioning is technically difficult
X-ray imaging	1 μm	high resolution; enables μm to multicentimeter mapping of anoxic features	resin embedding is technically difficult
Simulated			
no-flow/capillary fringe	multicentimeter	represents unsaturated systems	difficult to obtain adequate liquid sample volume
flow-through reactors	multicentimeter	controlled advection dynamics	limited applicability to unsaturated systems
microfluidic systems	micrometer	enables fine-scale measurements	abstracted from environmental conditions

potentially more likely to support the formation of elemental S, metastable FeS, and associated byproducts.[145] Finally, the concept of gradient magnitude provokes interesting questions regarding the transport of sulfidic byproducts into and through oxic environments. For example, metal sulfide clusters generated in sulfidic conditions[170] are remarkably resistant to oxidation and were found to contribute to contaminant transport in oxic rivers.[171] By logical extension, anoxic microsites in oxic aquifers could release metal sulfide clusters to oxic environments, but how far these aqueous metal sulfide clusters can migrate away from their generation point, i.e., the interior of an anoxic microsite, remains unknown.

In sulfate-rich soils, long periods of anoxia can promote the formation of Fe−sulfides, which, when oxidized, generate acidity. Although we typically associate acid sulfate soils with flooding and draining wetlands, presumably these acidification dynamics can occur at a smaller spatial scale. For example, if a persistent, sulfidic anoxic microsite were to become oxic, local acidification would transpire, which in turn alters soil weathering and nutrient, contaminant, and OC mobility. Regular redox fluctuations in sulfidic soils can drive pulses of contaminant release with subsequent cycles releasing more contaminant metals depending on the substrate.[172−174]

4. TECHNIQUES FOR CHARACTERIZING ANOXIC MICROSITES

The net influence of anoxic microsites on soil biogeochemistry is still largely unknown due, in part, to methodological constraints on identifying and characterizing anoxic microsites. Here, we evaluate existing approaches for detecting anoxic microsites and assessing their distribution, redox gradient magnitude, and temporality. We categorize these techniques into (1) bulk approaches that indicate anoxic microsite

presence and contribution to biogeochemical processes, (2) spatially explicit 2D imaging approaches, and (3) simulated systems (Table 1).

4.1. Bulk and 1D Measurements. Many bulk soil measurements can determine whether anoxic microsites are present and reveal some aspects of their distribution and gradient magnitude. In general, bulk and 1D measurements tend to be easier and cheaper to implement than 2D measures. Bulk and 1D measurements commonly used to identify anoxic microsites include (i) soil O_2 and redox state, (ii) concentrations of redox-active chemical species, and (iii) characteristics of soil microbial communities.

4.1.1. Oxygen and Soil Redox State. Oxygen concentrations offer a direct and instantaneous measure of anoxic microsites. Early evidence for the existence of anoxic microsites consisted of O_2 distribution measurements within wet aggregates of a silt loam soil using O_2 microelectrodes,[36] and this line of work was later carried out in combination with measurements of soil redox potential (Eh).[175] In more recent work, Hall et al. (2016) used O_2 sensors in the field to measure variations in bulk O_2 concentration semicontinuously for multiple months.[176] However, bulk O_2 content does not always reflect the spatial heterogeneity of biogeochemical conditions. For example, Keiluweit et al. (2018) confirmed the presence of anoxic microsites with 2D planar optodes, but bulk O_2 measurements showed consistently oxic conditions.[51] Similarly, in the field, Teh et al. (2005) consistently observed methanogenesis in soils which, when measured by bulk O_2 probes, were consistently oxic.[85] As a result, bulk O_2 measurements in a field or laboratory setting can result in "false negatives" in identifying anoxic microsites.

Soil redox potential (Eh) has also been used to characterize anoxic microsites in the field and laboratory. Flessa and Beese

(1995) used redox microelectrodes to characterize changes in redox potential across depth,[118] and more recently, redox electrodes were used to identify functionally different microsites in soils and overall redox heterogeneity.[48] Installing redox electrodes is relatively simple and inexpensive. However, Eh measurements can be difficult to interpret.[177] Like O_2 probes, redox probes make stationary point measurements and, therefore, can miss anoxic microsites in space. Additionally, depending on the size of the redox probe, Eh values reflect an integrated average over some volume, which can obfuscate microsites smaller than the probe resolution. Finally, Eh values need to be interpreted alongside pH in order to deduce the redox conditions.[178]

To avoid the issues associated with point measurements, mediated amperometric approaches allow characterization of the overall redox state of a soil on bulk samples, enabling the averaging of redox state over a confined soil volume. Mediated amperometry enables quantification of electron-accepting and -donating capacities of soil. These capacities represent the cumulative response of redox-active compounds in the samples, including solid-phase electron acceptors and their reduced species, quinone/hydroquinone groups in organic matter, and S^{2-}. Aeppli et al. (2022) used this approach to characterize spatiotemporal variations in soil redox state in a simulated aquifer system.[179] However, unlike Eh and O_2 measures, mediated amperometry has yet to be successfully deployed in a field setting.

4.1.2. Redox-Active Chemical Species. Gaseous byproducts of anaerobic respiration can also provide evidence for anoxic microsites. For instance, CH_4 has been used as an indicator for methanogenesis in well-oxygenated soils in the field[85,180,181] and laboratory.[37,51,85,182,183] Similarly, N_2O emissions may be used as an indicator for anoxic microsites.[118] Although N_2O and CH_4 fluxes are useful real-time indicators for anoxic microsites, both can be difficult to interpret on their own. Methane produced in anoxic microsites can be consumed by methanotrophs elsewhere in the soil profile, obfuscating any detectable signal at the soil surface. Nitrous oxide can be produced via multiple pathways, both abiotic (e.g., chemodenitrification)[107,140] and biotic (e.g., nitrification and denitrification).[184] Thus, N_2O fluxes are not necessarily indicative of denitrification. To address these ambiguities, authors have used isotopic approaches to attribute N_2O to specific (de)nitrification pathways[107,140,185,186] and to detect CH_4 production even when net CH_4 flux from soils is negative or zero.[37,181] Alternatively, others have used the co-occurrence of net CH_4 oxidation and N_2O reduction as an indicator for the presence of anoxic microsites.[187]

Dissolved and solid-phase measurements can also reveal anoxic microsites. Iron and Mn reduction can be monitored by measuring Fe(II) and Mn(II) in the dissolved, adsorbed, or solid phase.[51,67,79] Similarly, S^{2-} formed during microbial SO_4^{2-} reduction can be monitored in the dissolved phase or solid phase through precipitation of iron–sulfide minerals.[65] Whereas measuring solid-phase Fe(II), Mn(II), and S^{2-} often requires more advanced and costly X-ray techniques (see section 4.2.3), monitoring dissolved or extractable species is relatively simple and low cost with several methods performed colorimetrically.[188−190] Thus, when appropriate, many opt for dissolution protocols (e.g., Fe with weak hydrochloric acid) coupled with subsequent dissolved-phase measurements (e.g., Ferrozine for Fe(II), iodine titration for S^{2-}, and/or mass spectrometry when appropriate). Of course, there are

challenges to measuring and interpreting Fe(II), Mn(II), and S^{2-}. For example, Fe(II) readily reacts with O_2 to form Fe(III),[191] and aqueous sulfides react to form hydrogen sulfide, which escapes soil but can be detected as H_2S gas.[192] Mn(II) is relatively stable under oxic conditions but can be oxidized biotically to Mn(IV). As a result, Fe(II), Mn(II), and S^{2-} can represent both present and near-past anoxic conditions.

Although the products of specific redox reactions can indicate which biogeochemical transformations are occurring in soils and sediments, these chemical species should not be used as sole indicators of anoxic microsites. For example, a soil may host anoxic microsites that support Fe and Mn reduction but fail to support denitrification due to insufficient NO_3^- and/or fail to support sulfate reduction or methanogenesis due to insufficient redox decline. In this case, relying on N_2O, CH_4, or S^{2-} as the only indicators for anoxic microsites could conceal anoxic microsite presence.

4.1.3. Microbial Analyses. The presence of facultative or obligate anaerobes can provide strong evidence for present or historical anoxic (micro)environments. Common microbial analyses require isolation of DNA and/or RNA from approximately 0.25 to 5 g of soil. Minimum DNA and RNA extraction mass limits the ability to spatially resolve micrometer- or even millimeter-scale anoxic microsites but does allow for relative comparisons of anoxic microsite distribution. Analyses of DNA and RNA require different interpretations. DNA represents the metabolic potential of the microbial community and is relatively long lived in soils,[193,194] whereas RNA is ephemeral and represents present microbial function.[195] As a result, the presence of anaerobe DNA indicates metabolic potential for soil anaerobiosis in the past or present, whereas anaerobe RNA indicates active anaerobes at the time of sampling.[196]

Amplicon sequencing, typically of 16S rRNA subunits, yields the genetic makeup of the microbial community and their relatedness to known isolates. Through these associations and given the conserved nature of dissimilatory anaerobic respiration pathways within specific microbial taxonomic groups, "community analysis" or "molecular fingerprinting" of soil microorganisms can be used to determine whether a sample contains anaerobes and, therefore, anoxic microsites.[179,197,198] However, sequencing alone does not specifically target active cells or microbial functions.[199] Sequencing must be used in conjunction with bioorthogonal noncanonical amino acid tagging (BONCAT) coupled with fluorescence-activated cell sorting (FACS)[200] or stable isotope probing (SIP) with labeled ^{13}C and ^{15}N substrates or even $H_2^{18}O$ to link microbial community composition to functional traits (e.g., growth rate, death rate, substrate use efficiency).[201−203]

Quantification of target genes, typically those associated with anaerobic metabolisms, may be better suited than 16S rRNA sequencing to confirm and compare the presence of anoxic microsites. Quantitative polymerase chain reaction (qPCR) compares the amplification of a gene of interest using specific primers—for instance, primers targeting enzyme components involved in denitrification or methanogenesis—against amplification of a standard to yield gene copy number of that target on a soil mass basis.[46,113,179] The emerging digital (dPCR) and digital droplet PCR (ddPCR) instruments enhance the sensitivity of the method, improving the detection sensitivity of small microbial populations like methanogens in upland soils.[72] Notably, ddPCR does not require specimen standards, which can be laborious or even impossible to obtain.[204]

Depending on the quality of the nucleic acid extracts, resolution in environmental samples can be poor for both qPCR and (d)dPCR.[205]

Finally, a more exploratory approach to characterize microbial community functional potential and activity is through shotgun sequencing of whole-soil DNA or RNA samples to yield metagenomic and metatranscriptomic data.[206] Metagenomic techniques offer a glimpse into the whole soil functional potential, such as respiratory pathways, growth history, transporters, and other traits relevant to microbial lifestyle, and the addition of metatranscriptomics suggests microbial investment in these functions.[38,180,207] As a diagnostic method of anaerobiosis, however, these techniques suffer from high computational and analytical costs. Microarray chips that use nucleic acid probes attached to fluorophores offer a less comprehensive but functionally similar way to identify specific functional genes or taxa and quantify them via fluorescence upon hybridization of isolated and digested sample DNA or RNA-derived cDNA with the complementary sequence for the gene of interest (e.g., "GeoChip" as in refs 208 and 209).

4.2. Two-Dimensional Measurements. In general, spatially resolved measurements of relatively undisturbed soil allow us to define anoxic microsite distribution and gradient magnitude more clearly. Most of the bulk techniques described above require some destruction of soil structure, undermining the applicability of findings to natural systems. Two- and three-dimensional measures allow researchers to maintain soil structure while revealing patterns about the location and size of microsites and the degree of redox decline within anoxic microsites. To our knowledge, no 3D soil imaging techniques directly reveal the absence of O_2 nor the stable byproducts of anaerobic processes. So, although 3D techniques like computed tomography (CT) and 3D OrbiSIMS can provide useful information on soil properties that lead to or emerge from anoxic microsite formation,[210−212] we do not discuss 3D techniques here.

4.2.1. Planar Optode Measurements. Planar optode systems have enabled the measurement of multiple chemical species over space and time. Oxygen-sensing planar optodes have successfully revealed anoxic microsite presence and development primarily in laboratory settings.[51,63,213,214] Hydrogen sulfide and Fe(II) planar optodes are less commonly used, in part, because these sensors tend to be irreversible (i.e., single use), limiting their reusability and ability to provide time-resolved measures.[215] Practical constraints limit the use of planar optodes in a field setting,[216] and inserting a "wall" into 3D soil structure alters diffusion dynamics, potentially misrepresenting the in situ behavior of anoxic microsites.[215]

4.2.2. Spatially Resolved Microbial Techniques. Opportunities to identify and characterize anoxic microsites through spatially resolved microbial techniques are limited. Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) is a reliable and flexible technique that allows for the identification of functional genes or microbial biomarkers in 2D. During CARD-FISH, a reporter-labeled oligonucleotide probe binds to a complementary DNA segment in a 2D mounted sample. The binding of the probe activates the fluorescent label, and a confocal laser microscope is used to view and record the signal of the fluorescent probe. Tyramide signal amplification in catalyzed reporter deposition (CARD) significantly improves signal-to-noise fluorescence intensity.[217] Yet, CARD-FISH still has significant limitations for identifying

and characterizing anoxic microsites. First, 2D CARD-FISH is limited to analyses of small, thin slices of sample, which requires some disruption of soil structure and makes the technique difficult to implement on larger samples. Second, it can be difficult or even impossible to discern fluorescent tags from naturally fluorescent soil particles, even with the additional fluorescence provided by the tyramide signal.[217]

4.2.3. X-ray Fluorescence Imaging Combined with Spectroscopy. Synchrotron X-ray methods may be used to detect solid-phase evidence of anoxic microsites, past or present. Synchrotron-based X-ray fluorescence yield 2D imaging and spectroscopy methods provide high chemical sensitivity (fluorescent X-rays can be detected at very low signal strength) and the ability to map micrometer-scale redox contrasts for a large subset of elements, including S, Fe, Mn, As, U, Cr, and Mo.[17,18,34−36] X-ray 2D imaging and spectroscopy can reveal clusters of reduced, solid-phase elements which are interpreted to signify past or present anoxic microsites. X-ray 2D imaging and spectroscopy is particularly well suited for the study of anoxic microsites in its ability to bridge data across scales. Anoxic microsites can be mere micrometers in diameter,[51] requiring equally small spatial resolution for detection; yet, it is also necessary to quantify the spatial distribution of these microsites in a larger context, such as along multicentimeter soil cores. X-ray 2D imaging can provide spatial resolution down to ~1 μm[218] and measurements on samples tens of centimeters long, such as lengthwise soil core slices (up to 100 cm long)[219] or 30 cm × 20 cm rhizoboxes.[220]

Because X-ray 2D imaging and spectroscopy tracks the byproducts of anoxic microsites and not the lack of O_2 itself, X-ray 2D imaging and spectroscopy is an inherently indirect technique. However, depending on which element(s) are mapped, X-ray 2D imaging and spectroscopy methods can provide information on current, recent, and/or past anoxic conditions in the sample's history.[65] For example, detection of Fe(II) clusters would indicate anoxic microsites present at or close to the time of sampling. Alternatively, detection of lepidocrocite, the oxidized, stable product of Fe(II)−minerals and elemental S, would indicate that Fe(II)−sulfides and therefore anoxic microsites were present at some point in time but not at the time of sample collection.

Like other 2D methods, X-ray imaging and spectroscopy is well suited to reveal anoxic microsite distribution but comes with logistical shortcomings. As with planar optodes, proper X-ray imaging requires a relatively flat surface. Thus, intact soil cores must be cut for imaging. Slicing intact soil cores is technically difficult, may result in smearing or loss of particles, and can introduce O_2. Cores can be cast in epoxy to stabilize particles and diminish the risk of oxidation upon cutting, but the penetration of epoxy is limited in fresh (moist/wet) soil, making it difficult to ensure even epoxy casts. Further, synchrotron analyses are time intensive, and accessibility is limited.

4.3. Simulated Experimental Systems. One way to get around the considerable technical difficulties in detecting and characterizing anoxic microsites and their influence is to construct laboratory analogues of varying complexity, such as no-flow/capillary-fringe incubation reactors,[51,221−223] diffusion-limited flow reactors,[67,68,224−227] flow-through columns/reactors with dual-flow regimes,[166,168,179,226,228] and microfluidic systems.[214,229−231] In these systems, distinct redox zones of various dimensions and composition are created by

permeability contrasts (e.g., by inserting a diffusion-limited aggregate/lens within an advection dominated domain[166,168,179,226,228]). Within these distinct zones, anoxic microsites are established in response to variation in flow and exchange rates in reactors[67,68,221,225−227] or microfluidic systems.[229] Biogeochemical factors in these experimental systems are measured over time and space to obtain detailed information about types and rates of reactions inside anoxic microsites and exchange flows and rates across redox boundaries and gradients. Experimental systems are also well suited to investigate system responses to manipulation, such as changes in flow rates, influent water chemistry, or water saturation. Despite limitations arising from creating a simplified system (compared to nature), the knowledge gained from well-designed simulated systems is invaluable as presently it is extremely difficult if not impossible to obtain dependable measures of anoxic microsites in the field. Simulated experiments, therefore, serve as a critical link between field measurements and numerical models by elucidating key mechanisms and reaction rates in systems with anoxic microsites.[167,229]

5. MODELING ANOXIC MICROSITES

Modeling anoxic microsites remains a challenge. Classical methods for modeling redox processes constrain biogeochemical function through a series of equations drawn from soil physics, chemistry, and biology. These equations are then solved for discrete points in time using singular values for soil moisture content, O_2 concentration, redox potential, and microbial kinetics, among other parameters.[232−234] These classical methods allow for representations of temporal variability in soil biogeochemistry but assume that soil properties are spatially constant (Figure 3).[232] As a result, classical methods cannot adequately model the spatiotemporal variability of anoxic microsites. Nevertheless, there have been several promising advances in the modeling of anoxic microsites.

Spatially discretizing soil into distinct biogeochemical environments represents a relatively simple approach to modeling anoxic microsites. For example, Currie (1961) discretizes soils into aggregate and interaggregate pores with distinct O_2 diffusion coefficients for each pore category.[15] Parkin (1987) tediously separated soil volumes, measuring the denitrifying enzyme activity and denitrification rates of discretized units to construct a bimodal classification of high denitrification or minimal denitrification soil volumes.[64] Others have called for the discretization and modeling of anoxic microsites based on aggregates and aggregate size[44] and/or distinct soil environments, such as rhizosphere or subsoils.[233] One shortcoming of spatial discretization is the oversimplification of redox conditions; it is unlikely that soil moisture, O_2 concentrations, and/or denitrification capacities are truly bimodal or categorical throughout an entire soil volume (Figure 3).

To overcome some of the shortcomings that result from treating soils like distinct biogeochemical units, models that integrate probability distribution (or density) functions allow for continuous representations of soil spatial heterogeneity. Instead of representing key soil parameters as single values, probability distribution (or density) functions represent soil parameters as a continuous distribution of values, which are informed and constrained by soil measurements (Figure 3). For example, in an extension of the classical dual-Arrhenius
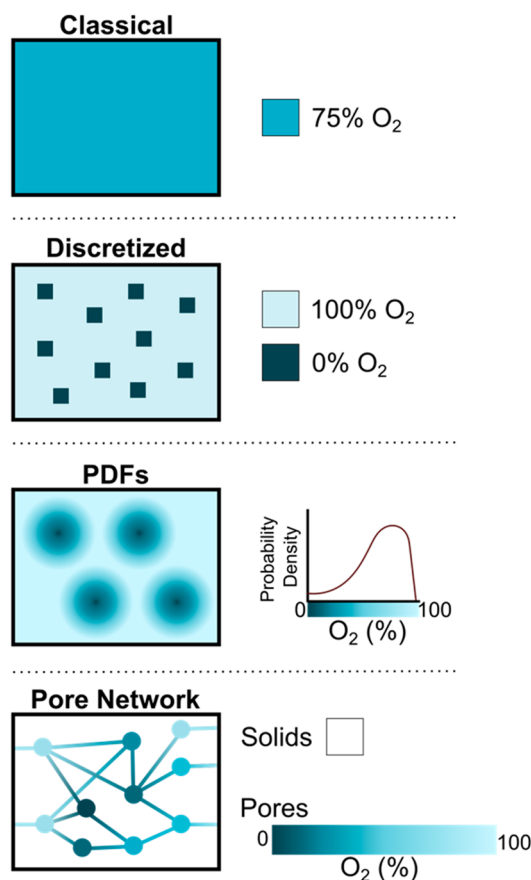

Figure 3. Depiction of different modeling approaches for representing anoxic microsites. Each rectangle represents a confined soil volume with shades of blue representing different oxygen (O_2) concentrations to represent redox heterogeneity. In theory, terminal electron acceptors other than O_2 can also be represented using these same approaches. PDFs = probability distribution (or density) functions.

Michaelis−Menten model, Sihi et al. (2020) represented soil OC content and moisture as skewed distributions with low probability of high-moisture and high-OC contents.[187] Instead of using single values in the network of equations represented in the dual-Arrhenius Michaelis−Menten model, distributions of OC and moisture were used to produce a distribution of outcomes, such as CO_2 efflux, which were used to predict subsequent distributions of other soil variables, such as O_2 concentration, N_2O, and CH_4 fluxes.[187]

Finally, other models more explicitly represent soils as porous media through which water and O_2 move while sustaining biogeochemical transformations. Ebrahimi and Or (2015) developed a 3D angular pore network model to characterize anoxic microsite distribution, lifetime, and response to hydration conditions within a single aggregate.[235] The 3D angular pore network model was later expanded to include interactions of aggregates of different sizes and orientations[50] and upscaled to demonstrate how aggregate size distributions alter anoxic microsite distribution and, thus, biogeochemical gas fluxes from soil profiles.[236] Pore-scale modeling of anoxic microsites is often paired with X-ray microcomputed tomography (μCT), which can constrain values for diffusivity and water distribution.[96,237] Larger scale models, based on laboratory experiments, have accurately represented anoxic microsites and their biogeochemical

influence in 2D using the principles of Darcian flow and reactive transport modeling.[167]

6. ACKNOWLEDGING AND APPRECIATING ANOXIC MICROSITES

While anoxic microsites often comprise only a small fraction of the total soil volume, they can have disproportionately large effects on the fate and transport of critical elements and compounds. However, decades after their first direct measurement,[36] anoxic microsites are not yet integrated into our mainstream conceptualizations of soil biogeochemistry. Water content is typically assumed to be the sole control on soil O_2 content,[238] with unsaturated soils tacitly accepted as entirely aerobic.[73] Even the Soil Science Society of America's *Methods of Soil Analysis* portrays anoxic microsites as anomalous, stating that "apparent bad readings" on redox probes "may be the microzone around the electrode [driving]... readings not representative of the bulk soil".[239] By continuing to overlook anoxic microsites, we pre-emptively accept the null hypothesis, H_0: Anoxic microsites have no effect on soil biogeochemistry.

In addition to acknowledging that anoxic microsites exist, we must appreciate the diversity that exists within the anoxic microsite classification, such as their varying distributions, redox gradients, and temporality. Connecting anoxic microsite features to soil properties will help reveal (i) the suite of factors that regulate anoxic microsite dynamics (i.e., the formation and persistence) and (ii) the influence of anoxic microsites on soil biogeochemistry. Filling both knowledge gaps is prerequisite to improve Earth System Models and project soils' responses to change. We envision a basic framework for advancing the study of anoxic microsites.

First, we recommend investing in our ability to measure the distribution and impact of anoxic microsites in soils. Presently, researchers must combine a bevy of techniques (Table 1) to confirm the presence and define the characteristics of anoxic microsites in soils and sediments. Furthermore, there is no reliable way to detect anoxic microsites in intact soils, and there are no unified metrics for anoxic microsite distribution, gradient magnitude, nor temporality. We suggest developing measures that (i) directly or indirectly quantify anoxic volume, (ii) characterize dominant electron-acceptor species across 2D or 3D space, and (iii) provide a sense of spatially resolved redox history, revealing anoxic microsites in real time as well as in the recent past. Ideally, these measures could be employed on intact soils. Developing and using these measures will help elucidate anoxic microsite distribution, forecast redox gradient-dependent reactions, and project fluxes of redox-sensitive elements over time.

Second, to hasten translation of information from measurements to models, robust, easily measurable, and scalable predictors of anoxic microsites should be defined. Repeating newly developed and/or refined measures of anoxic microsite characteristics across a variety of soils and ecosystems promises to reveal edaphic and/or climatic parameters that effectively predict anoxic microsite distribution, gradient magnitude, and temporality. These predictors can help project anoxic microsite dynamics across ecosystems, allowing more research to start linking anoxic microsite presence and traits to larger scale ecosystem function through field, laboratory, or modeling studies.

In brief, it is time to consider the anoxic microsite. Anoxic microsites exist and alter critical biogeochemical cycles. Ignoring anoxic microsites precludes needed technological

advances and studies of redox heterogeneity and biogeochemical cycling. Through identifying anoxic microsites, characterizing their variability, and quantifying their impact on various elemental cycles, we can advance our understanding of soils and improve our ability to project redox-sensitive elemental response to change.

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Notes

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