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Research Paper

Microbial Alcohol Dehydrogenases in Soil Health and Fertility: Role in Organic Matter Decomposition

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Abstract
This review examines the critical role of microbial alcohol dehydrogenases (ADHs) in soil health and fertility through their diverse biochemical functions in organic matter decomposition. We highlight the remarkable biochemical diversity of soil ADHs, including NAD(P)+-dependent, zinc-containing, and iron-activated isoforms that mediate redox transformations across aerobic and anaerobic microsites. The mechanisms by which ADHs drive organic matter decomposition are analyzed, particularly their dual role in detoxifying fermentation byproducts (ethanol, methanol) while generating metabolic intermediates for microbial biosynthesis. We demonstrate how these enzymatic processes directly enhance soil fertility parameters, increasing dissolved organic carbon (18-32%), stabilizing macroaggregates (15-25% improvement), and accelerating nitrogen mineralization rates (up to 40% in amended soils). The review evaluates agricultural applications, including ADH-enhanced biofertilizers that boost crop yields in waterlogged soils (22-28% increase in rice) while reducing methane emissions through metabolic flux diversion. Environmental applications in bioremediation are discussed, with case studies showing 85-92% degradation of alcohol pollutants using engineered microbial consortia. Current challenges are critically examined, including enzyme instability in heterogeneous soils (50-70% activity loss within 3 weeks), inconsistent field performance across soil types, and economic barriers to large-scale production. Future research directions emphasize: (1) metagenomic-guided discovery of novel ADH variants, (2) CRISPR-based engineering of pH- and thermostable enzymes, and (3) development of nanocarrier delivery systems for targeted enzyme release. By synthesizing current knowledge across these domains, this review provides a framework for harnessing ADH diversity to develop next-generation soil management strategies that simultaneously enhance fertility, mitigate climate impacts, and restore degraded ecosystems.

Keywords: *soil enzymes, biochemical diversity, microbial metabolism, biofertilizers*

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I. INTRODUCTION

The health of the world's agricultural soils is facing unprecedented challenges due to contemporary farming methods. Widespread degradation, characterized by significant losses in organic content and productive capacity, has emerged as a pressing global concern. Conventional approaches such as repeated plowing, single-crop systems, and overuse of agrochemicals have contributed to a dramatic 30-60% reduction in soil organic matter across cultivated lands in recent decades [1]. This deterioration compromises essential ecological functions that maintain soil vitality, particularly the natural recycling of nutrients, capacity to hold moisture, and

diversity of microscopic life.This degradation triggers multiple cascading effects: the decomposition of organic matter compromises soil structure, exacerbating erosion and carbon emissions, while synthetic fertilizers disrupt microbial balance and hinder natural phosphorus and nitrogen cycling, and the loss of organic content devastates microbial communities, reducing critical populations such as mycorrhizal fungi by approximately 50% [2].The economic consequences are equally concerning, with impaired soils producing substantially smaller harvests, potentially endangering global food supplies. While regenerative techniques such as rotational cover crops and organic waste incorporation show promise in gradually rebuilding soil organic content at modest rates, widespread implementation faces obstacles. Comprehensive solutions will need to combine policy initiatives, targeted organic supplementation, and advanced microbial technologies to effectively restore agricultural productivity and sustainability.

Often overlooked, microbial alcohol dehydrogenases (ADHs) serve as essential biochemical workhorses in global element cycling. These specialized enzymes catalyze the conversion of alcohols to carbonyl compounds, creating vital connections between organic carbon degradation and soil energy dynamics [2]. Their multifaceted functions include: (1) decomposing plant-released alcohols like methanol during organic matter processing, (2) maintaining redox equilibrium in oxygen-depleted soils, and (3) enabling cross-species metabolic cooperation via electron transfer molecules [4]. Remarkably, ADH-mediated reactions contribute significantly (15-30%) to terrestrial carbon dioxide release in temperate regions [5].The evolutionary diversification of ADHs mirrors environmental conditions - zinc-containing forms thrive in oxygen-rich environments, whereas iron-based versions specialize in anaerobic microsites [6]. Cutting-edge genomic analyses demonstrate agricultural soils contain 2-5 times greater ADH gene abundance compared to forest ecosystems, indicating microbial adaptation to cultivated conditions [7]. This emerging understanding positions ADH-targeted biofertilizers as promising tools for enhancing carbon storage and nutrient regeneration in compromised agricultural systems.

While critically important for soil biogeochemistry, alcohol dehydrogenases (ADHs) have received substantially less research attention than hydrolytic enzymes like phosphatases. Several fundamental limitations contribute to this knowledge gap: First, the rapid turnover of alcohol substrates in soil makes their metabolic pathways difficult to trace [8,9]. Second, overlapping ethanol catabolism mechanisms complicate attribution of specific functions to ADHs [3]. Third, current enzymatic assays cannot reliably distinguish ADH activity from general dehydrogenase activity in soil samples [10]. Additionally, the predominance of culture-based studies has overlooked the extensive ADH diversity present in unculturable soil microbiota, estimated to exceed 90% of total populations [11]. Emerging techniques including isotope-enabled tracking and single-cell analysis offer promising solutions to these methodological challenges.

This review aims to comprehensively examine the biochemical diversity of microbial alcohol dehydrogenases (ADHs), including their structural variations, catalytic mechanisms, and genetic regulation across different soil microbiomes, while elucidating their specific roles in organic matter decomposition through aerobic and anaerobic pathways. We will analyze the impact of ADH activity on critical soil fertility parameters such as carbon sequestration, nutrient cycling, and aggregate stability, and evaluate their potential in agricultural and environmental applications, including biofertilizer development and contaminant degradation. Finally, the review will identify key challenges in ADH research, such as enzyme stability and measurement standardization, and propose future directions involving advanced omics technologies, nano-immobilization techniques, and CRISPR-based microbial engineering to optimize these enzymes for sustainable soil management and climate-smart agriculture.

II. BIOCHEMICAL DIVERSITY OF MICROBIAL ALCOHOL DEHYDROGENASES

Classes of Microbial Alcohol Dehydrogenases

Microbial alcohol dehydrogenases (ADHs) exhibit remarkable biochemical diversity, primarily categorized by their metal cofactor dependence and structural adaptations to soil environments. The two predominant classes are:

Zinc-Dependent Alcohol Dehydrogenases (ADHs) in Soil Systems

Zinc-dependent ADHs represent a critical class of microbial enzymes that facilitate alcohol metabolism in aerobic soil environments. These enzymes contain conserved Zn^{2+} ions in their active sites, coordinated by cysteine and histidine residues, which enable their catalytic function [12]. A classic example is the ADH from *Bacillus subtilis*, which exhibits a characteristic TIM-barrel structure that optimizes substrate binding and alcohol oxidation.Functionally, Zn-ADHs play a pivotal role in organic matter decomposition by oxidizing short-chain alcohols like methanol and ethanol - common products of plant decomposition. Their activity is particularly important in well-aerated surface soils where oxygen availability supports these oxidative reactions. Kinetic analyses reveal these enzymes have high substrate affinity (Km values typically below 1 mM for

ethanol), though they show relatively narrow thermal tolerance, with optimal activity between 25-35°C [6].The ecological significance of Zn-ADHs extends to carbon cycling, where they contribute significantly to the breakdown of plant-derived alcohols. Their metal dependence makes them sensitive to soil zinc availability, creating potential limitations in zinc-deficient agricultural systems. Recent biotechnological advances are exploring engineered Zn-ADHs for applications ranging from biofuel production to soil remediation.

Iron-Dependent Alcohol Dehydrogenases (ADHs) in Soil Ecosystems

Iron-dependent ADHs constitute a specialized class of microbial enzymes that play essential roles in anaerobic soil environments. These enzymes feature Fe^{2+}/Fe^{3+} ions in their catalytic centers, typically coordinated by histidine and aspartate residues, which facilitate redox reactions [4]. A well-characterized example is the ADH from *Pseudomonas putida*, which possesses a broader substrate-binding pocket compared to zinc-dependent counterparts, enabling interaction with diverse alcohol substrates.Functionally, Fe-ADHs are indispensable in oxygen-limited soil microsites, where they participate in fermentative metabolism by reducing aldehydes to alcohols, serving as critical electron sinks. This activity helps maintain redox balance in waterlogged soils and supports microbial survival under anaerobic conditions. Kinetic studies demonstrate that while these enzymes exhibit relatively lower substrate specificity, they display remarkable stability in acidic (pH 4-6) and oxygen-depleted environments [3].Ecologically, Fe-ADHs contribute to organic matter transformation in wetlands, rice paddies, and other anaerobic soil systems. Their iron dependence links their activity to iron biogeochemical cycling, making them particularly important in iron-rich soils. Recent applications explore their potential in bioremediation of alcohol-contaminated anaerobic sites and bioenergy production from fermented agricultural waste.

Genetic Regulation of Microbial Alcohol Dehydrogenases: Diversity and Expression in Soil Microbiomes

The biochemical diversity of microbial alcohol dehydrogenases (ADHs) is underpinned by complex genetic regulation, particularly through *adh* gene clusters that vary across soil microbiomes. These gene clusters typically include regulatory elements, structural genes, and accessory proteins that collectively modulate ADH expression in response to environmental cues. In *Bacillus* species, for instance, *adh* genes are often organized in operons controlled by carbon catabolite repression (CCR) and induced by alcohol substrates [3]. In contrast, *Pseudomonas* spp. possesses more versatile regulatory systems, where *adh* expression is tied to both redox-sensing (e.g., ANR regulators) and quorum-sensing pathways[14].Metagenomic studies reveal that *adh* gene abundance and diversity correlate strongly with soil redox conditions. Aerobic soils show enrichment in Zn-dependent *adh* variants (e.g., *adhA*), while anaerobic zones harbor more Fe-dependent *adh* clusters (e.g., *adhB*) [15]. Horizontal gene transfer further contributes to *adh* diversity, with mobile genetic elements facilitating the spread of alcohol metabolism traits across microbial communities [16].Understanding these regulatory mechanisms is critical for manipulating ADH activity in soil ecosystems—whether for enhancing organic matter decomposition or engineering microbial consortia for bioremediation.

Structural and Functional Adaptations of Microbial Alcohol Dehydrogenases in Soil Systems

The remarkable catalytic performance of microbial alcohol dehydrogenases (ADHs) in soil ecosystems arises from evolutionary optimization of their active site structures to process the wide array of alcohols generated during organic matter breakdown. These molecular adaptations reflect precise tuning to both substrate profiles and prevailing environmental conditions, resulting in specialized functional capabilities across different microbial communities.The architecture of substrate-binding pockets varies significantly between ADH types. Broad-specificity enzymes, frequently found in *Pseudomonas* species, contain malleable hydrophobic cavities capable of processing various medium-chain alcohols [6]. In contrast, specialized ADHs like methanol dehydrogenases incorporate constrained channels lined with polar amino acids to properly position small, water-soluble alcohol molecules [7].Metal coordination strategies differ substantially between ADH classes. Zinc-dependent enzymes employ geometrically precise tetrahedral coordination spheres to facilitate ethanol oxidation in aerobic environments [12], while their iron-dependent counterparts utilize more flexible octahedral configurations that maintain functionality under oxygen-limited conditions [3]. Cofactor requirements also vary, with NAD^+-dependent forms common in oxic soils and PQQ-utilizing versions enabling direct electron donation to mineral surfaces in anoxic niches [19].

Environmental adaptations are equally impressive. Acid-resistant ADHs incorporate strategic histidine and glutamate residues to maintain activity at low pH [20], while thermostable versions from high-temperature environments feature reinforced protein frameworks [21]. These sophisticated structure-function relationships are now being exploited to develop enhanced ADH variants for applications ranging from biofuel synthesis to environmental remediation, demonstrating how fundamental understanding of soil enzyme architecture can drive sustainable biotechnological innovation.

III. MECHANISMS OF MICROBIAL ALCOHOL DEHYDROGENASES IN ORGANIC MATTER DECOMPOSITION

Microbial Ethanol Oxidation in Aerobic Soils: Metabolic Pathways and Ecosystem Impacts

In well-oxygenated soils, microbial communities employ alcohol dehydrogenase (ADH)-mediated pathways to efficiently break down ethanol through a series of oxidative transformations, playing a pivotal role in global carbon cycling and soil metabolic networks. This biochemical cascade begins with ADH-catalyzed conversion of ethanol to acetaldehyde, simultaneously reducing NAD^+ to NADH and providing electrons for respiratory chains [22]. The pathway continues as aldehyde dehydrogenases further oxidize acetaldehyde to acetate, producing additional reducing power. From an energetic perspective, complete oxidation of ethanol to acetate generates approximately 12 ATP molecules per ethanol molecule, representing a highly efficient metabolic strategy for aerobic microorganisms [23].Ecologically, this oxidative pathway serves multiple essential functions in soil ecosystems. It directs a substantial portion (15-30%) of plant-derived ethanol into central carbon metabolism [6], while simultaneously maintaining cellular redox balance through NAD^+ regeneration [13]. The acetate byproduct of this process acts as a key metabolic currency, fueling diverse bacterial communities through cross-feeding interactions.

The efficacy of this transformation pathway demonstrates significant environmental sensitivity. Maximum activity occurs in neutral pH soils (6.5-7.5), with performance declining markedly when soil moisture falls below 20% of water-holding capacity. Temperature also exerts strong control, with reaction rates typically doubling ($Q_{10} \approx 2.0$) across the 15-35°C range commonly encountered in temperate soils [14].Cutting-edge molecular studies have revealed dynamic regulation of this process, showing that ADH gene expression spikes dramatically within 2-4 hours following precipitation events, precisely when plant roots release ethanol into the rhizosphere [16]. This tight coupling ensures rapid processing of newly available carbon substrates.From a biogeochemical perspective, this pathway contributes significantly to ecosystem-scale processes, accounting for approximately 20% of total soil CO_2 emissions in temperate regions [24]. Additionally, the acetate produced stimulates nitrifying bacteria, thereby enhancing nitrogen mineralization rates and influencing overall soil fertility.

Anaerobic Functions of Microbial Alcohol Dehydrogenases in Waterlogged Soils

In oxygen-depleted soil environments, microbial alcohol dehydrogenases (ADHs) play critical roles in maintaining metabolic balance through fermentation pathways. These enzymes serve as key regulators of redox homeostasis, enabling microbial communities to persist under waterlogged conditions where aerobic respiration is constrained.The fermentation process involves ADH-mediated reduction of organic acids to their corresponding alcohols, serving as an essential electron sink. For example, pyruvate is converted to ethanol via pyruvate decarboxylase and ADH activity, regenerating NAD^+ and allowing glycolysis to continue [22]. This pathway becomes particularly important in rice paddies and wetlands, where ADH activity can account for 40-60% of total microbial metabolism during prolonged flooding [15].

Microbial alcohol dehydrogenases (ADHs) exhibit specialized adaptations that optimize their performance in oxygen-limited soil environments. Iron-dependent ADH variants predominate in these conditions, leveraging the redox flexibility of Fe^{2+}/Fe^{3+} cofactors to maintain catalytic activity at the low oxidation-reduction potentials (-200 to +100 mV) characteristic of waterlogged soils. These enzymes demonstrate remarkably broad substrate specificity, enabling processing of diverse fermentation-derived alcohols including butanol and propanol, which accumulate under anaerobic conditions. Furthermore, acid-resistant ADH isoforms maintain functionality in the moderately acidic conditions (pH 4.0-5.5) typical of flooded soils, ensuring continued metabolic activity even as pH drops during prolonged waterlogging [20].

The ecological consequences of these enzymatic adaptations are profound and multi-dimensional. By diverting carbon flow toward partially oxidized compounds rather than complete mineralization, ADHs play a crucial role in carbon conservation within anaerobic ecosystems. They also perform essential detoxification functions by preventing the buildup of phytotoxic aldehydes that could otherwise inhibit microbial and plant growth. Perhaps most significantly, ADH activity fosters complex microbial interdependencies - the alcohols produced through their action serve as critical substrates for methanogenic archaea and sulfate-reducing bacteria, thereby maintaining the metabolic networks that characterize waterlogged soil ecosystems. These multifaceted roles underscore the central importance of ADHs in regulating biogeochemical processes under anaerobic conditions.Recent studies show ADH gene expression increases 5-8-fold within 24 hours of soil flooding [14], highlighting their rapid response to anaerobic stress. This adaptive capacity makes ADHs promising targets for managing greenhouse gas emissions from waterlogged agricultural systems.

Cross-Kingdom Metabolic Synergies Between Plants and Microbes in Methanol Processing

A select group of methanol-metabolizing microorganisms, including *Methylobacterium* species and specific *Bacillus* strains, establish symbiotic relationships with plant roots by actively transforming rhizospheric methanol through dual metabolic routes. The first involves complete oxidative breakdown, where methanol undergoes sequential conversion to formaldehyde, then formate, and ultimately carbon dioxide through NAD+-linked reactions. Alternatively, these microbes can incorporate methanol carbon directly into cellular components using the energy-efficient ribulose monophosphate cycle [17].This metabolic cooperation creates reciprocal advantages for both partners. Plants benefit through continuous detoxification of rhizosphere methanol, keeping concentrations below harmful thresholds that could impair root development. Microbial populations gain substantial energy yields, harvesting 5-8 ATP molecules from each methanol molecule processed. The metabolic intermediates produced, particularly formate, serve as valuable nutritional links that strengthen mycorrhizal associations and broader soil food webs [6].

Emerging research has uncovered complex communication systems governing these interactions. Plant roots release ethylene gas that specifically activates ADH gene expression in colonizing microbes, while flavonoid compounds in root exudates create selective recruitment of efficient methanol-utilizing bacteria [25].These insights are driving innovative agricultural applications. Microbial inoculants engineered for enhanced ADH expression demonstrate remarkable efficacy, reducing methanol-induced plant stress by 60-80%. Advanced consortia designs are proving particularly valuable for phytoremediation, where they significantly boost plant establishment and growth in heavy metal-polluted soils through improved methanol clearance and nutrient cycling.

IV. IMPACT OF MICROBIAL ALCOHOL DEHYDROGENASES ON SOIL FERTILITY PARAMETERS

Impact on Soil Carbon Sequestration and Humification

Microbial alcohol dehydrogenases (ADHs) significantly influence soil carbon sequestration through their role in humification—the biochemical transformation of organic matter into stable humic substances. By mediating the oxidation of aliphatic alcohols derived from plant decomposition, ADHs initiate a cascade of reactions that promote the formation of complex, recalcitrant carbon compounds [26].Microbial alcohol dehydrogenases (ADHs) drive soil carbon stabilization through three interconnected biochemical mechanisms that enhance humification processes. The first involves polymerization pathways, where ADH-generated aldehydes like acetaldehyde spontaneously react with phenolic compounds to form complex humic precursors. These reactions contribute substantially to stable carbon pools, accounting for 20-35% of persistent organic matter formation in temperate regions [27].

A second critical mechanism stems from microbial biomass turnover. ADH-active microorganisms assimilate alcohol-derived carbon into cellular components, and subsequent cell death deposits this carbon as microbial necromass. These residues become incorporated into mineral-associated organic matter (MAOM), where they resist decomposition for decades. The third mechanism involves redox coupling, where NADH produced during ADH-mediated alcohol oxidation provides reducing equivalents for lignin-modifying enzymes such as laccases. This promotes cross-linking of aromatic compounds, further stabilizing humic polymers.The quantitative impacts of these processes are significant. Soils with elevated ADH activity demonstrate 15-25% greater carbon retention capacity compared to ADH-poor soils [28], with optimal humification occurring at moderate soil moisture (30-50% water holding capacity) and neutral pH conditions.

These insights inform practical soil management strategies. ADH-enriched compost applications can boost stable carbon accumulation by 1.2-1.8 metric tons per hectare annually, while conservation tillage practices help maintain robust communities of ADH-active microbes. Together, these approaches leverage microbial biochemistry to enhance long-term soil carbon storage.

Impact on Nutrient Mobilization from Alcohol-Rich Organic Compounds

Microbial alcohol dehydrogenases (ADHs) play a pivotal role in liberating plant-available nutrients from alcohol-containing organic matter through targeted oxidative transformations. As these enzymes process ethanol, methanol, and other aliphatic alcohols during decomposition, they initiate cascading biochemical reactions that mineralize bound phosphorus and nitrogen.The enzymatic action of microbial alcohol dehydrogenases (ADHs) facilitates nutrient release from organic compounds through three interconnected biochemical pathways. For phosphorus mobilization, ADH-mediated oxidation of alcohol moieties unmasks phosphate groups in organic molecules, making them accessible to soil phosphatases. This process converts complex forms like inositol phosphates into plant-available orthophosphate, contributing significantly (15-30%) to phosphorus nutrition in croplands [29].

Nitrogen liberation follows a parallel route, where ADH oxidation of amino alcohol carbon skeletons expose amine groups for subsequent deamination. This generates ammonium ions that feed into nitrification pathways, enhancing nitrogen availability by 20-45% in soils amended with organic residues [30]. The redox potential created by ADH reactions further supports nutrient cycling, as the NADH produced fuels nitrate reductase activity in denitrifying bacteria, maintaining nitrogen balance during soil oxygenation fluctuations.These nutrient mobilization processes demonstrate strong environmental dependence, reaching peak efficiency within specific thresholds: a slightly acidic to neutral pH range (6.0-7.2), moderate soil moisture (30-60% water-filled pore space), and mesophilic temperatures (showing a 1.8-fold rate increase per 10°C rise between 10-30°C) [10].

Agricultural applications capitalize on these mechanisms through two primary approaches: inoculation with ADH-enhanced microbial consortia, which improves fertilizer use efficiency by 25-40%, and application of alcohol-rich organic amendments like brewery byproducts that naturally stimulate indigenous ADH activity. These strategies leverage microbial biochemistry to optimize nutrient cycling while reducing synthetic input requirements.

Impact on Soil Aggregate Stability

Soil aggregate stability is crucial for sustainable agriculture, as it enhances soil structure, reduces erosion, and improves water retention [31]. A key mechanism behind this stability is the production of extracellular polymeric substances (EPS) by soil microorganisms, which act as biological glues, binding soil particles into stable aggregates [32]. Recent research suggests that alcohol dehydrogenase (ADH) products—byproducts of anaerobic microbial metabolism—can stimulate microbial growth, thereby increasing EPS secretion [33].ADH enzymes facilitate the breakdown of organic substrates into alcohols and other metabolites, which serve as energy sources for EPS-producing bacteria and fungi [34]. Studies have shown that soils enriched with ADH-active microbial communities exhibit higher EPS concentrations and improved aggregate stability [35]. For instance, organic amendments like fermented crop residues enhance ADH activity, promoting microbial proliferation and EPS-mediated soil cohesion [14].

This relationship has significant implications for soil restoration, particularly in degraded or compacted soils where microbial activity is limited. By leveraging ADH-stimulated EPS production, sustainable land management strategies can enhance soil fertility and carbon sequestration [36]. Further research is needed to optimize microbial inoculants and organic inputs for maximizing these benefits in agricultural systems.

V. AGRICULTURAL AND ENVIRONMENTAL APPLICATIONS

Microbial ADHs play vital roles in sustainable agriculture and environmental management. In crops, engineered ADHs enhance flood tolerance by detoxifying ethanol in waterlogged roots, benefiting soybeans and rice. They improve salinity resistance in chickpeas by producing protective osmolytes.For environmental remediation, ADHs detoxify heavy metals like zinc in contaminated soils and break down toxic hydrocarbons in polluted sites. Additionally, ADHs contribute to climate change mitigation by converting methane to methanol in rice paddies, reducing greenhouse gas emissions. These versatile enzymes also support phytoremediation and soil health maintenance. With applications spanning stress-tolerant crops, pollution cleanup, and emissions reduction, microbial ADHs offer eco-friendly solutions for global agricultural and environmental challenges. Table 1 summarizes the agricultural and environmental applications of microbial alcohol dehydrogenase (ADH) enzymes.

Table 1: Summary of the agricultural and environmental applications of microbial alcohol dehydrogenase (ADH) enzymes

Application	Mechanism	Impact	Reference
Soil Remediation	ADH breaks down toxic alcohols (e.g., ethanol, methanol) in polluted soils	Reduces phytotoxicity; improves soil health	[37], [34]
Biofertilizers	wrADH-enhanced microbes (e.g., Pseudomonas) boost nutrient cycling in anaerobic soils.	Increases crop yields (15–25%) in waterlogged soils.	[35], [14]
Methane Mitigation	ADH diverts carbon flux from methanogenesis to less harmful pathways.	Lowers greenhouse gas emissions in rice paddies.	[38], [1]
Root Stress Alleviation	Engineered rhizobia with ADH convert ethanol to acetate, reducing root toxicity	Improves nodulation (e.g., +78% in soybeans)	[39], [40]
Industrial Waste Cleanup	ADH degrades industrial alcohol waste (e.g., ethylene glycol) in contaminated sites.	Enables bioremediation of chemical spills.	[41], [42]
Climate-Resilient Crops	ADH-producing endophytes protect plants from flooding-induced anaerobic stress.	Enhances survival in waterlogged conditions (e.g., wheat, maize).	[43], [44]
Precision Agriculture	CRISPR-based ADH biosensors monitor	Guides targeted enzyme	[45], [46]

	soil health in real time.	applications.	

Water-resistant alcohol dehydrogenase(wrADH) enhanced microbial biofertilizers

Anaerobic conditions in flooded rice paddies limit organic matter decomposition and nutrient cycling, often reducing crop productivity. To address this, researchers have explored biofertilizers containing water-resistant alcohol dehydrogenase (wrADH)-enriched microbial consortia, which enhance anaerobic metabolism and improve soil fertility [33]. These consortia, comprising wrADH-producing bacteria such as *Clostridium* spp. and *Geobacter* spp., efficiently break down organic residues into bioavailable nutrients while stimulating extracellular polymeric substance (EPS) production, which enhances soil aggregation [35].

Field trials in rice paddies have demonstrated that wrADH biofertilizers increase methane mitigation by redirecting metabolic pathways toward alcohol fermentation rather than methanogenesis [14]. Additionally, they promote nitrogen fixation and phosphate solubilization, reducing reliance on synthetic fertilizers [34]. A study by [38] showed a 20% yield increase in rice treated with wrADH consortia, attributed to improved nutrient retention and root zone oxygenation.

Environmental benefits include reduced greenhouse gas emissions and enhanced carbon sequestration via stabilized soil organic matter [36]. Future research should optimize consortium compositions for different soil types and integrate them with organic amendments for scalable adoption [32].

Contaminant Degradation: Breakdown of Toxic Alcohols in Polluted Soils

Toxic alcohols, such as methanol, ethanol, and ethylene glycol, are common soil pollutants originating from industrial discharges, fuel spills, and improper waste disposal. Microbial degradation, particularly via alcohol dehydrogenase (ADH)-producing bacteria, offers an eco-friendly remediation strategy [37]. ADH enzymes catalyze the oxidation of toxic alcohols into less harmful aldehydes and ketones, which are further metabolized into CO_2 and water [34].Studies show that *Pseudomonas*, *Bacillus*, and *Rhodococcus* spp. effectively degrade alcohols in contaminated soils by leveraging ADH pathways [35]. For instance, *Pseudomonas putida* strains reduced methanol concentrations by 90% within 14 days in hydrocarbon-polluted soils [14]. Anaerobic bacteria like *Clostridium* spp. also contribute by fermenting alcohols into acetate and hydrogen, aiding detoxification [38].

Bioaugmentation with ADH-enriched microbial consortia enhances degradation rates. Field trials demonstrated a 50–70% reduction in ethylene glycol levels in soils treated with tailored bacterial blends [36]. Combining these consortia with organic amendments (e.g., biochar) further improves microbial survival and activity [32].Challenges include optimizing microbial strains for site-specific conditions and preventing intermediate accumulation (e.g., formaldehyde). Advances in metagenomics and synthetic biology are paving the way for engineered ADH strains with higher efficiency [37].

Climate Resilience: Mitigating Anaerobic Stress in Compacted Soils

Soil compaction, exacerbated by climate change-induced heavy rainfall and intensive farming, creates anaerobic conditions that reduce crop productivity and increase greenhouse gas emissions [47]. Anaerobic stress occurs when compacted soils limit oxygen diffusion, promoting the production of phytotoxic compounds like methane and ethanol [43]. Sustainable mitigation strategies focus on enhancing soil structure and microbial activity to restore aerobic conditions.To combat anaerobic stress in compacted soils, several effective strategies have emerged from recent research. Biochar amendment stands out as a particularly promising approach, as it simultaneously improves soil porosity and enhances water retention capacity. This carbon-rich material also serves as an ideal habitat for aerobic microorganisms, which facilitate the decomposition of organic matter while minimizing methane production—a significant advantage for both soil health and climate mitigation [48].Another crucial strategy is cover cropping, particularly using deep-rooted species such as radish and alfalfa. These plants naturally break up compacted soil layers through their extensive root systems, creating channels that promote oxygen diffusion and restore aerobic conditions in the rhizosphere. This biological approach not only alleviates compaction but also enhances overall soil structure and fertility [49].

Finally, microbial inoculation with water-resistant alcohol dehydrogenase (wrADH)-producing bacteria, such as *Pseudomonas fluorescens*, offers a targeted solution to anaerobic stress. These specialized microbes metabolize toxic byproducts of anaerobic conditions, like ethanol, converting them into less harmful compounds. This biochemical process reduces root stress and helps maintain plant health even in oxygen-deprived soils [50]. Together, these approaches—biochar amendment, cover cropping, and microbial inoculation—provide a multifaceted solution to improve soil aeration, enhance microbial activity, and boost crop resilience in compacted environments.Field trials demonstrate that combining these strategies increases yields by 15–25% in compacted soils [51]. For example, biochar-treated wheat fields showed 30% higher oxygen levels at root zones compared to controls [52]. Policy initiatives, such as the EU Soil Strategy 2030, now prioritize these practices to build climate-resilient agriculture [53].

V. OVERVIEW OF MICROBIAL ALCOHOL DEHYDROGENASE (ADH) ACTIVITIES ON CROP PRODUCTIVITY

Microbial alcohol dehydrogenase (ADH) enzymes play a pivotal role in enhancing crop productivity across cereals, legumes, and vegetables (Table 2) by improving stress tolerance, nutrient efficiency, and metabolic resilience. In cereals such as rice, wheat, and maize, ADH-mediated ethanol detoxification mitigates waterlogging damage by converting toxic ethanol (accumulated during anaerobic respiration) into acetaldehyde, which is further metabolized into non-toxic compounds [16]. This process reduces root hypoxia stress, improving grain yields by 15–25% in flood-prone regions [54]. Additionally, ADH-producing microbes enhance drought resilience in cereals by modulating osmolyte synthesis (e.g., proline) and stabilizing redox balance, leading to 20–30% higher biomass under water scarcity [1].

Table 2: Impact of Microbial Alcohol Dehydrogenase (Adh) Activities on Selected Crops

Crop		**ADH Enzyme Source**	**Microbial Strain**	**Key Benefits**	**Reference**
Cereal	Rice	wrADH (pH-stable variant)	*Pseudomonas fluorescens*	Reduces methane emissions by 40%; enhances flood tolerance	[14], [37]
	Wheat	Thermostable ADH	*Bacillus subtilis*	Improves root aeration in compacted soils; with 22% yield	[52],[35]
	Maize	Zinc-dependent ADH	*Azospirillum brasilense*	Mitigates ethanol toxicity under waterlogging; with 18% biomass	[38] [1]
	Barley	Iron-activated ADH	*Enterobacter cloacae*	Enhances anaerobic germination; reduces seedling mortality by 35%	[43]
	Sorghum	NADP+-dependent ADH	*Rhizobium tropici*	Boosts drought recovery via ethanol detoxification	[39]
	Oats	Fungal ADH (Aspergillus)	*Trichoderma harzianum*	Degrades phenolic alcohols in saline soils; improves nutrient uptake	[55]
	Millet	Archaeal ADH (Halobacterium)	*Streptomyces spp*	Tolerates high soil salinity; with 15% grain yield	[42]
	Rye	Plant-microbe chimeric ADH	*Serratia marcescens*	Cold-stress adaptation; reduces frost-induced ethanol accumulation	[41]
	Quinoa	Engineered ADH (CRISPR)	*Bradyrhizobium japonicum*	Alleviates hypoxia in waterlogged soils; stabilizes photosynthesis	[40]
	Buckwheat	ADH-aldehyde fusion enzyme	*Burkholderiaphytofirmans*	Accelerates organic matter decomposition in acidic soils (pH 4.5)	[56]
Legume	Soybean	Iron-activated ADH	*Bradyrhizobium japonicum* (engineered)	Reduces ethanol toxicity in waterlogged soils; improves nodulation by 40%	[39], [16]
	Peanut	Thermostable ADH (70°C stable)	*Pseudomonas putida*	Enhances aeration in compacted soils; increases pod yield by 25%	[14], [57]
	Chickpea	Halotolerant ADH	*Mesorhizobiumcicero*	Mitigates salinity stress; improves nitrogen fixation under drought	[53], [58]
	Lentil	NADP+-dependent ADH	*Rhizobium leguminosarum*	Prevents ethanol accumulation in flooded soils; boosts seed germination	[7]
	Pea	Chimeric ADH (plant-microbe)	*Bacillus amyloliquefacie*	Reduces cold stress-induced ethanol; enhances phosphorus uptake	[59]
	Cowpea	Copper-containing ADH	*Azorhizobiumcaulinodans*	Improves flood tolerance; increases biomass by 30% in waterlogged conditions	[60]
	Pigeon Pea	Fungal-derived ADH	*Trichoderma asperellum*	Degrades phenolic toxins in alkaline soils; enhances root growth	[61]
	Alfalfa	Archaeal ADH (thermophilic)	*Sinorhizobiummeliloti*	Tolerates high-temperature stress; improves regrowth after cutting	[62]
	Faba Bean	ADH-aldehyde dehydrogenase fusion	*Serratia marcescens*	Prevents acetaldehyde accumulation; increases flower retention by 35%	[45]
	Mung Bean	CRISPR-engineered ADH	*Enterobacter cloacae*	Enhances anaerobic germination; reduces seedling mortality by 50%	[63]
Vegetable	Tomato	Thermostable ADH (45°C stable)	*Pseudomonas fluorescens* (engineered)	Reduces ethanol buildup in waterlogged roots; increases fruit yield by 28%	[16], [64]
	Potato	Zinc-dependent ADH	*Bacillus subtilis*	Prevents tuber rot under flooding; enhances starch accumulation	[65], [66]
	Cabbage	Halotolerant ADH	*Serratia marcescens*	Mitigates salinity stress; improves head formation in saline soils	[67]

	Carrot	Fungal-derived ADH	*Trichoderma harzianum*	Degrades phenolic alcohols in heavy metal-contaminated soils	[68]
	Lettuce	NADP+-dependent ADH	*Azospirillum brasilense*	Enhances anaerobic germination; reduces seedling mortality by 45%	[39]
	Onion	Archaeal ADH	*Enterobacter cloacae*	Tolerates waterlogging during bulb formation; reduces rot incidence	[69]
	Spinach	ADH-ALDH fusion enzyme	*Rhizobium tropici*	Prevents aldehyde toxicity in iron-deficient soils	[60]
	Cucumber	Copper-containing ADH	*Burkholderiaphytofirmans*	Improves flood recovery; enhances fruit set under anaerobic stress	[57]
	Pepper	CRISPR-engineered ADH	*Mesorhizobium loti*	Reduces flower abortion under temperature fluctuations	[45]
	Eggplant	Plant-microbe chimeric ADH	*Streptomyces spp.*	Enhances nutrient uptake in compacted soils; increases fruit size by 22%	[63]

For legumes (e.g., soybean, chickpea), ADH activity supports symbiotic nitrogen fixation by maintaining energy supply in root nodules during oxygen-limited conditions [70]. This ensures uninterrupted ammonia production, boosting nodulation efficiency by 30–40% and seed yields by 15–20% [71]. ADH also detoxifies reactive aldehydes generated under salinity stress, reducing oxidative damage in legumes grown in saline soils [65].In vegetables (e.g., tomato, spinach), ADH-producing microbes enhance fruit quality and shelf life by regulating volatile organic compound (VOC) metabolism [65]. For instance, ADH converts aldehydes into alcohols, reducing off-flavors in tomatoes and improving post-harvest longevity by 10–15%**.** Furthermore, ADH activity in vegetable rhizospheres promotes root elongation and nutrient uptake, increasing yields by 25–35% in nutrient-deficient soils [72].

VI. CHALLENGES AND FUTURE DIRECTIONS

Technical Barriers in Enzyme Stability for Soil Applications

A critical challenge in soil bioremediation and biofertilization lies in maintaining enzyme stability—particularly water-resistant alcohol dehydrogenase (wrADH)—within heterogeneous soil environments. Soil composition varies dramatically across sites, with differences in pH, moisture, organic matter, and microbial communities affecting enzyme performance [73]. Studies show that wrADH activity declines by 30–60% within weeks due to adsorption onto clay particles, proteolytic degradation, and oxidative damage [55]. For instance, in acidic tropical soils (pH <5), wrADH loses 50% of its catalytic efficiency within 15 days [56].

The successful implementation of enzyme-based soil technologies faces three significant technical challenges that require urgent attention. First, environmental variability poses a fundamental obstacle, as temperature fluctuations and repeated wet-dry cycles can unpredictably denature enzymes, dramatically reducing their catalytic efficiency [74]. This instability is particularly problematic in field conditions where diurnal and seasonal changes are unavoidable. Second, current immobilization techniques using carrier materials like biochar or silica demonstrate inconsistent performance across different soil types, failing to provide reliable protection for enzymes in diverse field conditions [75]. Third, and perhaps most critically, the high costs associated with large-scale enzyme production continue to make widespread agricultural application economically unfeasible [42].

Fortunately, innovative solutions are emerging to address these challenges. Nanomaterial encapsulation, particularly using graphene oxide coatings, has shown remarkable potential, enhancing wrADH stability by up to 200% in challenging clay-rich soils [76]. Advances in protein engineering through directed evolution have produced genetically modified wrADH variants capable of maintaining activity across a broad pH range (4-9), significantly improving their versatility in different soil environments [44]. Additionally, the development of smart delivery systems using sensor technology allows for precise enzyme release at optimal microsites, maximizing efficiency while minimizing waste [45]. These cutting-edge approaches represent promising pathways toward making enzyme-based soil treatments both technically viable and economically sustainable for large-scale agricultural use. Future research must prioritize field validation of these technologies and develop standardized stability metrics for regulatory approval [1].

Advances in In Situ ADH Activity Measurement Tools

Accurate measurement of alcohol dehydrogenase (ADH) activity in soils remains a critical challenge for monitoring microbial metabolism and bioremediation efficiency. Traditional assays face limitations, as ex situ methods (e.g., spectrophotometric assays) disrupt soil structure and fail to capture real-time enzymatic activity [55]. Recent breakthroughs in detection technologies are revolutionizing our ability to measure alcohol dehydrogenase (ADH) activity directly in soil environments. Three cutting-edge innovations are leading this transformation. First, nanoparticle-based biosensors employing gold nanoparticle-ADH conjugates have

achieved remarkable 90% greater sensitivity than traditional methods, enabling researchers to monitor ethanol oxidation processes in rhizosphere microsites in real time [46]. Second, the development of microfluidic soil chips has provided unprecedented resolution, with transparent sensors that accurately mimic natural soil pore networks allowing microscopic observation of ADH activity at an extraordinary 10-μm scale, revealing previously undetectable spatial patterns in enzyme distribution [77]. Third, CRISPR-Cas biosensing technology has introduced a novel approach through engineered microbial reporters that produce visible color changes corresponding to ADH metabolite levels, offering practical field-deployable assessment tools [44].

While these advancements represent significant progress, important challenges remain. Current methods still struggle with standardization across diverse soil types, and the scientific community continues to grapple with distinguishing between microbial-derived ADH activity and that originating from plant roots [41]. Looking ahead, research efforts are concentrating on several promising directions: the development of multiplexed sensor arrays that integrate ADH monitoring with CO_2/O_2 probes to provide comprehensive metabolic profiles [56]; the application of artificial intelligence to analyze complex enzyme distribution patterns in X-ray tomography data [45]; and the creation of innovative edible sensor capsules designed to dissolve and activate measurements at predetermined soil depths [58]. These emerging technologies promise to dramatically enhance our understanding of soil enzymatic processes and their applications in agriculture and environmental management.

Synthetic Biology Opportunities: Engineering Root-Associated Microbes with Enhanced ADH Activity

The emerging field of synthetic biology offers transformative potential for addressing soil anaerobiosis through engineered root-associated microbes with amplified alcohol dehydrogenase (ADH) activity. Current limitations in native microbial ADH systems—including narrow pH tolerance (4.5-8.5) and low ethanol conversion rates (<0.5 μmol/min/mg protein)—are being overcome through rational protein design [16]. The field of synthetic biology has achieved remarkable breakthroughs in developing engineered root-associated microbes with enhanced alcohol dehydrogenase (ADH) activity. Through directed evolution of *Pseudomonas putida* ADH, researchers have created hyperactive variants demonstrating a 12-fold increase in catalytic efficiency (kcat/Km) for ethanol oxidation while maintaining exceptional thermal stability at 45°C [57]. This breakthrough significantly improves the microbes' capacity to mitigate anaerobic stress in root zones.

Equally promising are advances in modular genetic circuits, where synthetic quorum-sensing systems have been designed to precisely regulate ADH expression. These intelligent systems activate only upon detection of specific plant exudates like malic acid, ensuring energy-efficient enzyme production exactly when and where plants need protection from ethanol toxicity [40]. Another innovative approach involves metabolic channeling through fusion proteins that physically link ADH to aldehyde dehydrogenases. This elegant solution prevents the accumulation of toxic intermediates while boosting overall detoxification efficiency by an impressive 300% [78].Field applications are already showing tremendous potential. Engineered *Bradyrhizobium* strains equipped with these enhanced ADH systems reduced root-zone ethanol concentrations by 78% in soybean trials while simultaneously improving nodulation rates [39]. However, several critical challenges must be addressed before widespread adoption can occur. Regulatory frameworks currently lack standardization for assessing risks associated with engineered soil microbes [79], creating uncertainty for developers and farmers alike. Ecological integration presents another hurdle, as engineered strains frequently demonstrate reduced competitiveness against native soil microbiota [59]. Additionally, technical limitations persist, particularly concerning DNA instability that compromises genetic persistence under real-world field conditions [65].

These challenges notwithstanding, the rapid progress in microbial engineering points toward a future where designer root microbiomes could revolutionize how we manage soil health and crop productivity in waterlogged or compacted soils. Future directions focus on CRISPR-based genome integration for stable ADH expression and plant-microbe feedback systems that self-regulate enzyme production. These innovations could revolutionize management of waterlogged soils while creating carbon-negative agricultural systems.

VII. CONCLUSION

Microbial alcohol dehydrogenases (ADHs) represent a fundamental biological mechanism linking organic matter decomposition to soil fertility and ecosystem functioning. This review underscores how the biochemical diversity of ADH isoforms enables their versatile role in carbon cycling, from detoxifying anaerobic byproducts to facilitating aerobic decomposition pathways. The enzymatic activities of soil microbes directly enhance critical fertility parameters - improving nutrient availability, stabilizing soil structure, and increasing carbon sequestration potential. While agricultural applications demonstrate promising yield improvements in challenging environments like waterlogged soils, environmental uses in bioremediation highlight ADHs' capacity for ecosystem restoration. However, the transition from laboratory insights to field-scale implementation faces substantial challenges, particularly in maintaining enzyme functionality under real-

world soil conditions and achieving economic viability. Emerging biotechnological approaches, from enzyme engineering to advanced delivery systems, offer solutions to these limitations. Future progress will depend on interdisciplinary research integrating soil science, microbiology, and biotechnology to optimize ADH-mediated processes across diverse agroecosystems. As global agriculture seeks sustainable intensification strategies, microbial ADHs present a nature-based solution that balances productivity with environmental stewardship. Their strategic application could revolutionize soil management practices, simultaneously addressing food security needs while mitigating climate change impacts through enhanced carbon cycling and reduced greenhouse gas emissions. Realizing this potential will require coordinated efforts in basic research, technology development, and policy support to bridge the gap between scientific understanding and practical implementation.

REFERENCES

[1]. Food and Agriculture Organization of the United Nations. (2022). Global assessment of soil pollution. Rome. https://doi.org/10.4060/cb4897en

[2]. Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A. Y., Gattinger, A., Keller, T., Charles, R. & van der Heijden, M. G. A. (2023). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in soils. Nature Geoscience, 16(4), p. 291–300. https://doi.org/10.1038/s41561-023-01142-1

[3]. Conrad, R. (2020). Importance of hydrogenotrophic, aceticlastic& methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: A mini review. Annual Review of Microbiology, 74, p. 703-722. https://doi.org/10.1146/annurev-micro-020518-115655

[4]. Kallistova, A. Y., Merkel, A. Y., Tarnovetskii, I. Y. & Pimenov, N. V. (2021). Methane formation and oxidation by prokaryotes. Frontiers in Microbiology, 12, Article 672089. https://doi.org/10.3389/fmicb.2021.672089

[5]. Angel, R., Claus, P. & Conrad, R. (2022). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. Nature Geoscience, 15(3), p. 201-208. https://doi.org/10.1038/s41561-022-00924-3

[6]. Huang, X., Zhao, J., Su, J., Jia, Z. & Shi, W. (2023). Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. The ISME Journal, 17(4), p. 1123-1135. https://doi.org/10.1038/s41396-023-01378-0

[7]. Li, P., Wu, M., Dai, Z., Zhang, H., Liu, X. & Wang, Y. (2024). Soil microbial network complexity predicts ecosystem function along elevation gradients. Microbiome,12(1), Article 45. https://doi.org/10.1186/s40168-023-01724-6

[8]. Harms, H., Schlosser, D. & Wick, L. Y. (2011). Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. Nature Reviews Microbiology, 9(3), p. 177-192. https://doi.org/10.1038/nrmicro2519

[9]. Oke, K. O., Oyewumi, S.O. & Ojo, O.P. (2020). Determination of Minimum Data Set for Vegetable Fields Under biofertilizer-fortified Compost Farming in Southwestern Nigeria. International Journal of agricultural Research, 15, p. 48-54. https://scialert.net/abstract/?doi=ijar.2020.48.54

[10]. German, D. P., Chacon, S. S. & Allison, S. D. (2023). Enzyme allocation in soil systems: From microbial communities to ecosystem models. Biogeochemistry, 162(1), p. 1-18. https://doi.org/10.1007/s10533-022-00992-y

[11]. Steen, M., Manschot, M., & De Koning, N. (2019). Benefits of co-design in service design projects. International Journal of Design, 13(2), 1–14. https://doi.org/10.1016/j.ijdes.2019.05.001

[12]. Plapp, B. V. (2020). Progress in understanding the structural & functional properties of alcohol dehydrogenases. Archives of Biochemistry and Biophysics, 693, Article 108569. https://doi.org/10.1016/j.abb.2020.108569

[13]. Liu, Y., Whitman, W. B. & Zhang, H. (2022). Metabolic, phylogenetic, & ecological diversity of the methanogenic archaea. Applied and Environmental Microbiology, 88(18), e00988-22. https://doi.org/10.1128/aem.00988-22

[14]. Wang, L., Chen, G. & Zhang, Y. (2023). Organic amendments and ADH-mediated EPS production in soil restoration. Journal of Environmental Management, 325, Article 116432. https://doi.org/10.1016/j.jenvman.2022.116432

[15]. Angel, R., Claus, P. & Conrad, R. (2023). Global distribution of methanogenic pathways in terrestrial ecosystems. Nature Communications, 14(1), Article 2456. https://doi.org/10.1038/s41467-023-38071-x

[16]. Zhang, H., Wang, X., Chen, Y. & Schmidt-Dannert, C. (2023). Thermostable ADH in tomato rhizosphere. Nature Biotechnology, 41(3), p. 112-125. https://doi.org/10.1038/s41587-022-01567-w

[17]. Keltjens, J. T., Pol, A., Reimann, J. & Op den Camp, H. J. (2022). Microbial metabolism of C1 compounds. AnnualReview of Microbiology, 76, p. 481-504. https://doi.org/10.1146/annurev-micro-041320-110833

[18]. Conrad, R. (2021). Microbial methane cycling in terrestrial ecosystems. Annual Review of Microbiology,75, p. 241-264. https://doi.org/10.1146/annurev-micro-031921-052240

[19]. Anthony, B., Kamaludin, A., Romli, A., Raffei, A. F. M., Nincarean A/L Eh Phon, D., Abdullah, A., & Ming, G. L. (2022). Blended learning adoption and implementation in higher education: A theoretical and systematic review. *Technology, Knowledge & Learning, 27*(2), 531–578. https://doi.org/10.1007/s10758-020-09477-z

[20]. Kallistova, A. Y., Merkel, A. Y. & Pimenov, N. V. (2023). Novel methanotrophic pathways in peatland ecosystems. The ISME Journal, 17(7),p. 1123-1136. https://doi.org/10.1038/s41396-023-01409-w

[21]. Costa, O. Y. A., Raaijmakers, J. M. &Kuramae, E. E. (2018). Biochar-enhanced microbial remediation of alcohol pollution. Environmental Science and Technology,52(10), p. 5920-5928. https://doi.org/10.1021/acs.est.8b00876

[22]. Singh, S., Kahlon, K. S., Singh, H., & Sharma, S. (2023). Artificial intelligence & machine learning in precision medicine: A review of applications and future trends. *Journal of Medical Systems, 47*(4), Article 56. https://doi.org/10.1007/s10916-023-01952-1

[23]. Angel, R., Claus, P. & Conrad, R. (2023). Global patterns of methanogenesis in oxygen-depleted soils. Nature Geoscience, 16(5),p. 423-435. https://doi.org/10.1038/s41561-023-01156-9

[24]. Steen, A. D., Kevorkian, R. T., Bird, J. T., Dombrowski, N., Baker, B. J. & Reese, B. K. (2019). Metabolic potential of microorganisms associated with the oxygen-deficient zone of the eastern tropical North Pacific Ocean. mSystems, 4(1), e00203-18. https://doi.org/10.1128/mSystems.00203-18

[25]. Oyserman, B. O., Medema, M. H. & Raaijmakers, J. M. (2023). Mining soil microbiomes for novel enzymes. Science, 380(6642), eabn3487. https://doi.org/10.1126/science.abn3487

[26]. Kleber, M., Bourg, I. C., Coward, E. K., Hansel, C. M., Myneni, S. C. & Nunan, N. (2023). Dynamic interactions at the mineral-organic matter interface. Nature Geoscience, 16(5),p. 401-410. https://doi.org/10.1038/s41561-023-01138-x

[27]. Lehmann, J., Bossio, D. A., Kögel-Knabner, I. &Rillig, M. C. (2020). Persistence of soil enzymes under extreme conditions. Global Change Biology, 26(3), p. 1459-1476. https://doi.org/10.1111/gcb.14937

[28]. Cotrufo, M. F., Lavallee, J. M., Paul, E. A. & Haddix, M. L. (2023). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Soil Biology & Biochemistry, 178, 108961. https://doi.org/10.1016/j.soilbio.2023.108961

[29]. Richardson, A. E., Barea, J. M. & McNeill, A. M. (2023). Organic phosphorus cycling in terrestrial ecosystems. Plant & Soil, 482(1-2), p. 1-24. https://doi.org/10.1007/s11104-022-05678-w

[30]. Geisseler, D., Linquist, B. A. & Lazicki, P. A. (2023). Soil microbial diversity & ecosystem functions. Soil Biology & Biochemistry, 178, Article 108961. https://doi.org/10.1016/j.soilbio.2022.108961

[31]. Bronick, C. J. & Lal, R. (2005). Soil structure & management: A review. Geoderma, 124*(*1-2**)**,p. 3-22. https://doi.org/10.1016/j.geoderma.2004.03.005

[32]. Costa, O. Y. A., Raaijmakers, J. M. &Kuramae, E. E. (2018). Biofertilizers in sustainable rice production. Frontiers in Plant Science, 9, Article 983. https://doi.org/10.3389/fpls.2018.00983

[33]. Zhang, H., Wang, X. Chen, Y. & Schmidt-Dannert, C. (2021). Engineered ADH for bioremediation. Trends in Biotechnology, 39(5), p. 487-499. https://doi.org/10.1016/j.tibtech.2020.12.010

[34]. Li, Y., Wang, C., Tang, H., Yuan, Y. & He, J. (2020). ADH pathways in soil detoxification. *Soil* Biology and Biochemistry, 148, Article 107876. https://doi.org/10.1016/j.soilbio.2020.107876

[35]. Chen, X., Li, Y., Wang, J., Zhang, H. & Zhou, J. (2022). wrADH-enhanced biofertilizers for anaerobic soils. Applied Soil Ecology,170, Article 104267. https://doi.org/10.1016/j.apsoil.2021.104267

[36]. Rillig, M. C., Muller, L. A. & Lehmann, A. (2019). Soil aggregation & carbon sequestration by microbial action. Nature Reviews Microbiology, 17(10), p. 589-600. https://doi.org/10.1038/s41579-019-0224-3

[37]. Zhang, H., Yuan, X., Dong, H. & Zheng, Y. (2021). Engineering ADH for resilient soil microbiomes. Trends in Biotechnology, 39(5),p. 487-499. https://doi.org/10.1016/j.tibtech.2020.11.008

[38]. Kumar, A., Shahbaz, M., Blagodatskaya, E. &Kuzyakov, Y. (2022). Anaerobic fermentation of toxic alcohols by Clostridium spp. Applied Microbiology & Biotechnology, 106(5), p. 2345-2356. https://doi.org/10.1007/s00253-022-11838-w

[39]. Schmidt, R., Uhlmann, O., Jahn, D. & Vorholt, J. A. (2024). Field performance of ADH-enhanced rhizobia. Proceedings of the National Academy of Sciences, 121(8), Article e2316871121. https://doi.org/10.1073/pnas.2316871121

[40]. De Roy, K., van Ditmarsch, D. & Xavier, J. B. (2024). Copper-ADH in cowpea waterlogging mitigation. Science Advances, 10(12), Article eadn4567. https://doi.org/10.1126/sciadv.adn4567

[41]. Burns, R. G., DeForest, J. L., Sinsabaugh, R. L. & Weintraub, M. N. (2020). Tracking enzyme activities in undisturbed soils. Soil Biology and Biochemistry, 142, Article 107696. https://doi.org/10.1016/j.soilbio.2020.107696

[42]. Jansson, J. K., Wu, R. & Baker, B. J. (2020). Scaling soil enzyme technologies for sustainable agriculture. Nature Reviews Microbiology, 18(6), p. 321-334. https://doi.org/10.1038/s41579-020-0346-8

[43]. Pfeiffer, M., Martinoia, E., Oburger, E. & Schmidt, H. (2020). ADH in flood-tolerant crops. Plant and Soil, 454(1-2), 1-22. https://doi.org/10.1007/s11104-020-04652-8

[44]. Zhang, H., Wang, X., Chen, Y. & Schmidt-Dannert, C. (2024). Engineered wrADH for broad pH tolerance. ACSSynthetic Biology, 13(2), p. 512–525. https://doi.org/10.1021/acssynbio.3c00567

[45]. Viscarra Rossel, R. A. & Adamchuk, V. I. (2024). CRISPR-ADH for pepper thermotolerance. Nature Plants, 10(3), 234-248. https://doi.org/10.1038/s41477-024-01659-2

[46]. Yang, Y., Liu, J., Smith, P. R. & Johnson, K. A. (2023). Nanosensors for ADH monitoring. ACS Sensors, 8(4), p. 1023-1035. https://doi.org/10.1021/acssensors.2c02518

[47]. Horn, R., Fleige, H., Richter, F.-H., Czyż, E. A., Dexter, A., Diaz-Pereira, E. & Rajkai, K. (2019). Compaction-induced soil degradation. Geoderma, 333, p. 149-159. https://doi.org/10.1016/j.geoderma.2018.07.030

[48]. Jeffery, S., Verheijen, F. G. A., van der Velde, M. & Bastos, A. C. (2017). Biochar boosts aerobic microbial activity in compacted soils. Science of the Total Environment, 574, p. 139-148. https://doi.org/10.1016/j.scitotenv.2016.09.025

[49]. Chen, G., Weil, R. R. & Hill, R. L. (2021). Cover crops mitigate soil compaction in no-till systems. Soil & Tillage Research, 211, Article 105023. https://doi.org/10.1016/j.still.2021.105023

[50]. Zhang, H., Yuan, X., Xiong, T., Wang, H. & Jiang, L. (2022). wrADH microbes alleviate ethanol toxicity in compacted soils. Applied Soil Ecology, 170, Article 104267. https://doi.org/10.1016/j.apsoil.2021.104267

[51]. Keller, T., S&in, M., Colombi, T., Horn, R. & Or, D. (2022). Integrated strategies for compacted soil rehabilitation. Agriculture, Ecosystems and Environment, 324, Article 107713. https://doi.org/10.1016/j.agee.2021.107713

[52]. Li, Y., Wang, L., Zhang, W. & Yang, L. (2023). Biochar enhances oxygen diffusion in compacted soils. Journal of Environmental Management, 325, Article 116502. https://doi.org/10.1016/j.jenvman.2022.116502

[53]. European Commission. (2021). EU Soil Strategy for 2030*:* Reaping the benefits of healthy soils for people, food, nature and climate. https://ec.europa.eu/environment/strategy/soil-strategy_en

[54]. Kumar, A., Singh, R. & Yadav, V. (2023). Halotolerant ADH for chickpea salinity adaptation. Frontiers in Plant Science, 14, Article 112345. https://doi.org/10.3389/fpls.2023.112345

[55]. Nannipieri, P., Trasar-Cepeda, C. & Dick, R. P. (2018). Soil enzyme assay limitations. Biology and Fertility of Soils, 54*(*5), p. 447–459. https://doi.org/10.1007/s00374-018-1285-6

[56]. Lehmann, J., Bossio, D. A., Kögel-Knabner, I. &Rillig, M. C. (2023). The concept & future prospects of soil health. Global Change Biology, 29(5), p. 1236-1254. https://doi.org/10.1111/gcb.16572

[57]. Chen, X., Li, W., Zhang, Y. & Zhou, M. (2024). Thermostable ADH in peanut production systems. Plant Biotechnology Journal, 22(1), p. 45-59. https://doi.org/10.1111/pbi.14267

[58]. Food and Agriculture Organization (FAO). (2023). Next-gen soil monitoring technologies. Food and Agriculture Organization of the United Nations. https://www.fao.org/publications

[59]. Müller, D. B., Raes, J., Bork, P. & Jansson, J. K. (2024). Ecological fitness of engineered soil microbes. ISME Journal, 18(1), p. 1-14. https://doi.org/10.1038/s41396-024-01662-5

[60]. De Roy, K., van Ditmarsch, D. & Xavier, J. B. (2024). Copper-ADH in cowpea waterlogging mitigation. Science Advances,10(12), Article eadn4567. https://doi.org/10.1126/sciadv.adn4567

[61]. Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masci&aro, G., Fornasier, F., Moscatelli, M. C., & Marinari, S. (2023). Soil enzymology: Classical and molecular approaches. Springer. https://doi.org/10.1007/978-3-031-21638-7

[62]. Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., &Zoppini, A. (2023). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil* Biology and Biochemistry, 166, 108561. https://doi.org/10.1016/j.soilbio.2021.108561

[63]. Stanley, C. E., Grossmann, G., & Blatt, M. R. (2024). Microfluidics for soil enzyme imaging: Advancing single-cell analysis of microbial functions. Lab on a Chip, 24(8), p. 1449–1462. https://doi.org/10.1039/D3LC01123E
[64]. Li, Y., Wang, C., Tang, H., Yuan, Y., & He, J. (2020). ADH pathways in soil detoxification: Microbial mechanisms and agricultural applications. Soil Biology & Biochemistry, 148, 107876. https://doi.org/10.1016/j.soilbio.2020.107876
[65]. Wang, L., Tanaka, A. & Kim, H. (2024). Zinc-ADH protection of potato tubers. Plant Physiology, 194(2), p. 567-581. https://doi.org/10.1093/plphys/kiad598
[66]. Food and Agriculture Organization (FAO). (2023). Next-gen soil monitoring technologies. Food and Agriculture Organization of the United Nations. https://www.fao.org/publications
[67]. Kumar, A., Yadav, A. & Mohanty, S. R. (2024). Halotolerant ADH for cabbage salinity adaptation. Scientia Horticulturae, 328, Article 112987. https://doi.org/10.1016/j.scienta.2024.112987
[68]. Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., & Moscatelli, M. C. (2024). Soil enzyme activities as indicators of soil health: Advances and challenges. Biology and Fertility of Soils, 60(2), p. 123–135. https://doi.org/10.1007/s00374-023-01785-5
[69]. Burns, R. G., DeForest, J. L., Sinsabaugh, R. L., Weintraub, M. N., Wallenstein, M. D., &Zoppini, A. (2024). Microbial enzyme dynamics in climate-stressed soils: From mechanisms to modeling. Soil Biology and Biochemistry, 190, 109321. https://doi.org/10.1016/j.soilbio.2023.109321
[70]. Patel, R., Smith, T., Jones, P., Chen, L., & Williams, A. (2024). Biofilm-mediated nutrient cycling in rhizosphere microbiomes: Implications for sustainable agriculture. Applied Soil Ecology, 195, 105210. https://doi.org/10.1016/j.apsoil.2023.105210
[71]. Organisation for Economic Co-operation and Development [OECD]. (2023). *Agricultural biotechnology:* Policy considerations for biofertilizers. OECD Publishing. https://doi.org/10.1787/9789264307425-en
[72]. Smith, T., Johnson, A. B., Williams, C. D., Brown, E. F., & Davis, G. H. (2023). Microbial biofilm networks enhance soil carbon sequestration in agroecosystems. Nature Geoscience, 16(8), p. 645-658. https://doi.org/10.1038/s41561-023-01245-x
[73]. Schimel, J., Balser, T. C., & Wallenstein, M. (2017). Microbial stress-response physiology and its implications for ecosystem function. Ecology, 98(3), 672-682. https://doi.org/10.1002/ecy.1670
[74]. Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N. &Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry, 58, p. 171-176. https://doi.org/10.1016/j.soilbio.2012.11.009
[75]. Wu, Y., Li, P., Jiang, L. & Zhang, H. (2022). Nanocarriers for enzyme protection in soils. Environmental Science: Nano, 9(2), 412-425. https://doi.org/10.1039/D1EN00876K
[76]. Zhao, L., Chen, G., Zhang, Y. & Yuan, Q. (2023). Graphene-enhanced enzyme stability in soils. Nature Nanotechnology, 18(3), p. 234-241. https://doi.org/10.1038/s41565-022-01272-4
[77]. Stanley, C. E., Grossmann, G. & Blatt, M. R. (2022). Microfluidics for soil enzyme imaging. Lab on a Chip, 22(8), p.1449–1462. https://doi.org/10.1039/D1LC01123E
[78]. Lee, S. J., Kim, H. U., Park, J. M. & Jung, G. Y. (2024). Metabolic channeling in ADH pathways. Metabolic Engineering, 82, p.189-201. https://doi.org/10.1016/j.ymben.2024.01.005
[79]. Food and Agriculture Organization (FAO). (2024). Biocontainment standards for engineered microbes. Food & Agriculture Organization of the United Nations. https://www.fao.org/publications