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Review

# Soil Microbial Dynamics in Regenerative Agriculture Systems: A Data-Driven Synthesis for Soil Health, Pest Suppression, and Yield Sustainability in the Western Canadian Prairies

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## Abstract

Regenerative agriculture (RA) is expanding across the Western Canadian Prairies, but its microbial foundations under climatic constraint remain insufficiently integrated. This review synthesizes evidence from long-term Prairie field experiments, regional datasets and global meta-analyses to evaluate how regenerative management reshapes soil biological processes and system performance. Across studies, RA is consistently associated with increases in microbial biomass, enzymatic activity, arbuscular mycorrhizal connectivity, and nitrogen-use efficiency, alongside gains in soil organic carbon, aggregation, and water-holding capacity. These biological enhancements correspond with lower soilborne disease pressure, moderated weed dynamics, reduced dependence on synthetic nitrogen and pesticide, and progressively stabilized yields under semi-arid, short-season conditions. Prairie findings broadly align with global regenerative trends, although short-term and site-specific responses range from negative to positive, underscoring the importance of temporal scale. In regions characterized by high interannual climate variability, conserved microbial mechanisms appear central to resilience, while the rate at which agronomic benefits emerge depends on climatic and edaphic constraints. Overall, the synthesis identifies microbial restoration as the central pathway linking regenerative management to soil health, pest suppression, and sustainable productivity. Continued long-term, system-level research is needed to refine regionally adapted regenerative transitions and to clarify how microbial processes mediate resilience under future climate uncertainty.

**Keywords:** regenerative agriculture; reduced tillage; diversified rotation; cover cropping soil microbial dynamics; western Canadian prairies; soil health; pest suppression; yield sustainability

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## 1. Introduction

Soil microorganisms are pivotal to core processes that sustain agroecosystem, including nutrient cycling (e.g., nitrogen fixation, phosphorus solubilization), organic matter decomposition, soil water-retention, toxic compound degradation, the suppression of plant pests and pathogens [1]. These ecological processes underpin Regenerative Agriculture (RA), an outcome-driven, systems-based approach that integrates natural processes into farming to soil health and build sustainable, resilient production system systems [2]. Despite being the primary biological drivers of RA, soil microorganisms remain underemphasized and are often not well studied.

The consequences of this knowledge gap are particularly acute in the Canadian Prairie region, one of the world's largest contiguous agricultural landscapes and a major contributor to global food

security. The widespread adoption of high-yielding crop varieties, extensive monocultures, synthetic inputs, and mechanized farming dramatically increased food production but left a legacy of ecological disruption in Prairie agroecosystem [3]. Soil deterioration, organic matter and biodiversity loss, poor water management, and greenhouse gas emissions now threaten long-term sustainability and increase vulnerability to pest and disease outbreaks [4]. These pressures are intensifying under climate stress, as prolonged cold winters, relatively short growing seasons, recurring droughts, erratic precipitation and temperature extremes increasingly constrain yield stability across semi-arid to sub-humid Prairie zones [5]. In this context, understanding how microbial communities buffer or amplify these stresses has become central to designing effective RA strategies.

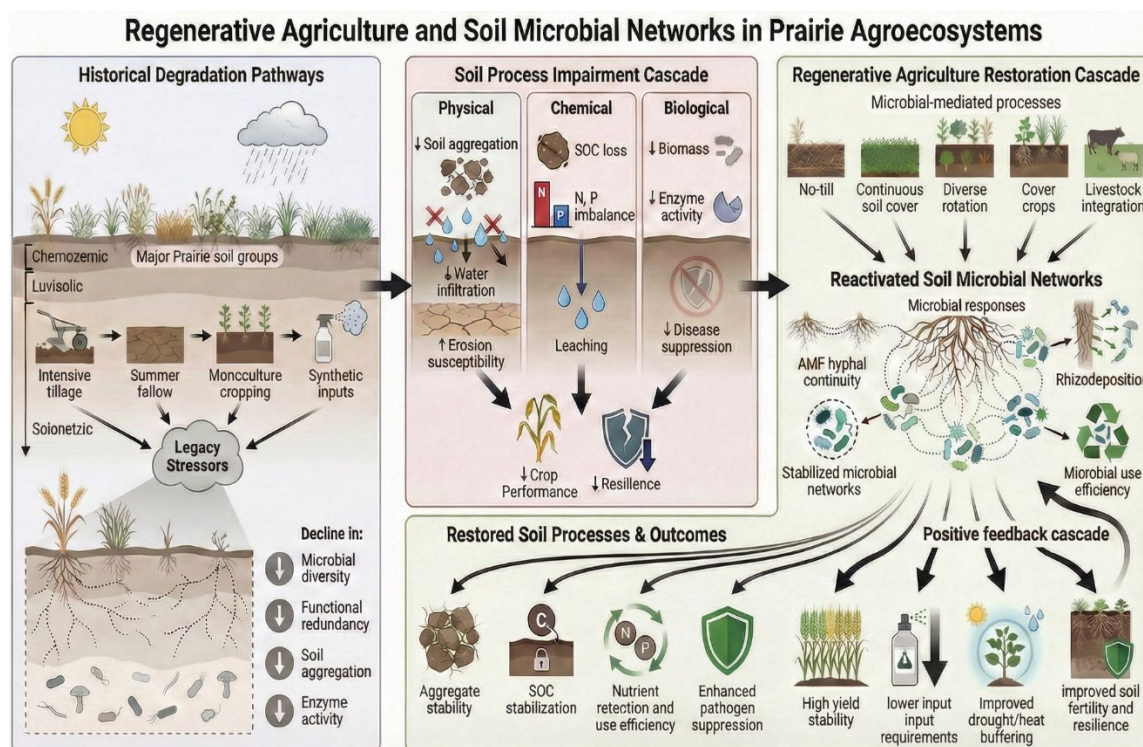
Prairie soils are dominated by Chernozemic, Luvisolic, and Solonchic orders, which are particularly sensitive to management because their structure, nutrient retention, and water-holding capacity depend strongly on organic matter and microbial activity. Agriculture and Agri-Food Canada's Soil Organic Carbon Change indicator reported that between 1981 and 2016, approximately 60% of prairie cropland experienced measurable SOC decline [6]. This pattern is consistent with site level analysis reporting SOC loss particularly where perennial grasslands were converted to annual cropping or wetlands were drained [7,8]. By contrast, lands with early adoption of no-till and diversified crop rotations showed SOC stabilization or modest recovery, indicating that degradation is management-driven rather than inevitable. These contrasting trajectories suggest that microbial process governing SOC and aggregation are highly responsive to how prairie soils are managed.

Sustainable practices such as cover cropping, reduced tillage, and holistic grazing management in the Canadian Prairies has been reported to rebuild soil organic matter, improve carbon sequestration, and thus contribute to reducing greenhouse gas emissions [4]. Adoption of RA practices has therefore emerged in the Western Canada not as a novelty but as a necessary corrective response, supported by government initiatives such as the Sustainable Canadian Agricultural Partnership [9]. Although Canada is not yet the global leader in conservation tillage, approximately 65% of arable prairie land is under no-till systems [10]. Cover cropping has also gained momentum, with 2020 surveys reporting that 281 farmers across the Canadian Prairies cultivated over 41,000 ha of cover crops, despite regional climatic challenges [11]. Taken together, these trends underscore that farmer are already shifting disturbance, residue, and grazing regimes in ways that implicitly rely on microbial recovery, even if microbial responses are not explicitly measured.

With rising climate risk, input costs, and ongoing soil degradation, Prairie agriculture faces an urgent need for biologically grounded solutions. That can deliver both resilience and productivity. Yet most syntheses remain narrowly focused on individual practices such as no-till, without integrating how multiple RA practices jointly restructure microbial communities and the process they control. As a result, a comprehensive, data-driven understanding of how RA practices reorganize soil microbiomes-and how these microbiomes mediate soil health, pest suppression, and yield stability-remains lacking. This review addresses that gap by synthesizing long-term field studies, prairie-scale experiments, and meta-analyses to clarify how RA practices unlock microbial potential to stabilize yields and rebuild soil function. Specifically, we aim to: (i) synthesize data-driven evidence linking RA practices to microbially mediated improvements in key soil health indicators (e.g., SOC, aggregation, nutrient cycling); (ii) elucidate microbial mechanisms underpinning enhanced crop yield and stress resilience in Canadian prairie systems; (iii) evaluate how microbial regulation under RA contributes to weed, insect, and pathogen suppression; and (iv) identify research gaps and future directions for microbiome-informed regenerative agriculture tailored to Western Canada. We hypothesize that RA enhances yield stability and long-term sustainability in the Canadian Prairies primarily through the restoration of soil microbial diversity, functional activity, and network resilience, positioning microbes as the central driver through which management reshapes ecosystem regulation.

## 2. A Century of Pressure on the Prairie Breadbasket

The Canadian Prairie provinces, spanning Alberta (AB), Saskatchewan (SK), and Manitoba (MB), approximately encompass a vast region of 52.6 M ha, comprising 81% of Canada's agricultural land [12]. Producing over 80% of Canada's field crops, the Prairies are widely recognized as a global "breadbasket" [13]. However, as illustrated in Figure 1 more than a century of intensive tillage, summer fallow, monoculture cropping, and heavy agrochemical use, compounded by growing climate stress, has progressively weakened soil structure, depleted soil organic carbon (SOC), and eroded agroecosystem resilience. Within this setting, the historical degradation pathways in Figure 1 frame the central question of this review: whether rebuilding soil microbial networks can shift Prairie agroecosystems from vulnerability toward recovery.



**Figure 1.** Conceptual framework for regenerative agriculture (RA) in Canadian Prairie agroecosystems. RA practices (reduced/no tillage, diverse rotations, cover crops, organic amendments, crop-livestock integration) counteract historical degradation by improving microbial habitat and networks, which in turn enhance soil structure, SOC stabilization, nutrient and water dynamics, pest suppression, and long-term yield stability while gradually lowering input needs.

### 2.1. Climate Constraints and Agronomic Vulnerability

Climatic constraints in the Canadian prairies place continual stress on soils and crops, making the region highly dependent on the buffering capacity of soil biota. The Canadian Prairie region experiences a continental climate with long winters, short growing seasons, and pronounced interannual variability in precipitation and temperature [14]. Recurrent droughts, heat waves, and episodic heavy rainfall increasingly not only affect crop productivity but also exacerbate soil degradation processes, such as erosion and moisture loss [13]. Between 2009 and 2019, severe weather events across Canada led to an average of \$1.9 billion annually in insured damage claims [13]. In Prairie systems, yield stability depends more on soil biological buffering capacity than on short-term inputs, a central message of Figure 1. RA practices minimize soil disturbance, improve infiltration, nutrient retention, consistently enhance microbially mediated processes which in turn mitigate climate risk by buffering soil moisture and temperature extremes rather than simply increasing mean yields. [12–14]. Thus, climate risk makes the microbial "buffering layer" depicted in Figure 1 not short-term inputs the critical target for regenerative management.

## 2.2. Soil Organic Carbon (SOC) Depletion and Legacy Effects

Historically, prairie soils supported high SOC stocks and diverse microbial communities due to native grasslands and deep perennial rooting systems. Twentieth-century agricultural intensification yield-maximization paradigm disrupted this equilibrium [15]. As depicted in Figure 1, repeated tillage, summer fallow, and land conversion caused widespread SOC loss, compaction, and salinity expansion, especially in fine-textured and marginal soils [15,16]. In Saskatchewan, the area under no-till expanded from just 8% in 1981 to 73% by 2016, contributing to a regional increase in SOC index values from 48 in 1981 to 78 in 2006 before stabilizing near 72 in 2016 [17,18]. Continuous wheat and summer fallow rotations in Swift Current, SK, lost up to 20-25% of SOC over three decades, compared with diversified rotations and perennial phases that maintained or slightly increased SOC [19,20]. These outcomes reinforce show that SOC decline is a legacy effect of disturbance rather than an inevitability and imply that RA strategies which rebuild microbial activity and carbon inputs can partially reverse this trajectory.

## 2.3. Monoculture and Biological Simplification

Economically successful but biologically simplified systems now dominate prairie agriculture, with cereals (e.g., wheat, barley), oilseeds (canola), and pulses (lentils, peas) occupying most cropped land [13]. While effective from a market standpoint, the practice of monocultures and intensive tillage in these farmlands led to biological homogenization and soil nutrient depletion [15]. Field studies show that cereal- or canola-dominated systems reduce microbial richness and favor generalist taxa adapted to low-diversity environments, while suppressing beneficial guilds such as arbuscular mycorrhizal fungi and antagonistic bacteria [15,21]. Reduced microbial niche availability limits functional redundancy, increasing vulnerability to disease outbreaks and climate stress. Thus, monoculture represents a microbial bottleneck that undermines long-term soil function and resilience in Prairie agroecosystems, and it motivates the RA focus on diversified rotations and continuous cover in Figure 1 as key levers for microbiome repair.

## 2.4. Reliance on Synthetic Input

A parallel trend has been the heavy reliance on synthetic nitrogen phosphorus, and pesticides, which has further reshaped Prairie soil ecosystems and their microbial communities. Excess nutrients favor fast-growing copiotrophic microbes while suppressing oligotrophic taxa that contribute to long-term carbon stabilization and nutrient-use efficiency [22,23]. Similarly, fungicides and herbicides can exert off-target effects on non-pathogenic fungi and bacteria, disrupting symbioses and reducing functional diversity [23,24]. In semi-arid Prairie soils, where SOC is already limited, this chemical dependence amplifies soil degradation, increasing reliance on external inputs and weakening microbial resilience. These patterns emphasize the need for management approaches such as RA that restore biological, especially microbial regulation of nutrients and pests rather than attempting to replace it indefinitely with chemical substitution.

## 2.5. Impacts on the Hidden Life Beneath Prairie Soils

Within this degraded and chemically dependent context, the “hidden” microbial life beneath Prairie soils emerges as a sensitive integrator of management and climate. Soil microbial activity is closely linked to soil moisture, temperature and organic inputs. Drought years consistently reduce microbial biomass and respiration, particularly among decomposers and nitrogen-cycling taxa [25]. Prolonged stress shifts communities toward stress-tolerant taxa, often reducing functional redundancy and ecosystem stability [26]. Lupwayi et al. (2001) found that conventional tillage caused greater microbial biomass losses in acidic, carbon-poor Luvisolic soils, than in carbon-rich Chernozemic soils, highlighting the buffering role of baseline SOC [27]. Across Prairie systems, tillage intensity often exerts a stronger influence on microbial community structure than fertilizer inputs, favoring opportunistic bacteria and pathogens over symbiotic fungi such as AMF [28]. Crops also

shape microbial communities through root exudates, residue inputs, and rotation patterns. Monocultures narrow community composition, whereas diversified rotations expand niche availability and support specialized guilds involved in nutrient cycling and disease suppression [29]. These observations align with Figure 1, positioning soil microbes as key mediators linking management decisions to long-term soil function and resilience, and they provide the mechanistic bridge to the next section, where regenerative practices are treated as deliberate attempts to re-engineer these microbial networks in prairie systems.

## 2.6. Framework of Prairie Regenerative Agriculture

In response to the long-term pressures outlined above, Prairie farmers and researchers have been applying principles now labelled “Regenerative Agriculture” since at least the 1970s, particularly through conservation tillage, residue retention, and crop diversification [30]. Guided by these six core principles (summarized in Table S 2.6), RA operates as a “stimulation-response-outcome” system; management practices stimulate microbial mechanisms, which drive ecosystem processes that ultimately shape agronomic and environmental outcomes.

Practices such as cover cropping, crop rotation, residue retention, and minimizing tillage effectively improve soil water-holding capacity, mitigate temperature extremes at root zone, increase SOC and soil aggregation, thus building Prairie climate resilience at the field scale [31]. Empirical studies have shown that regenerative fields exhibit greater microbial activity, higher water infiltration rates, and reduced nutrient leaching compared to conventionally managed lands [32]. In the Prairie context, crop diversification and crop rotation sequence significantly contribute to both soil fertility and disease regulation. Pulse-based rotations enrich nitrogen-fixing bacteria (*Rhizobium*, *Bradyrhizobium*), increase microbial biomass and diversity, and suppress certain soilborne pathogens, contributing to both fertility and disease regulation [33]. Deep-rooted and diverse crops further extend microbial habitat into subsoil layers, improving structure and resource access [34]. Collectively, these findings demonstrate that RA in the Canadian Prairies is a biologically grounded strategy for restoring soil function, buffering climate risk, and sustaining long-term productivity, with microbial networks, highlighted in Figure 1-acting as a central pathway through which these management changes translate into resilient agroecosystem outcomes.

## 3. Results

### 3.1. Effects of No-Till (NT) and Reduced Tillage (RT) on Soil

Table 3.1 summarizes long-term Prairie and temperate studies evaluating how no-till (NT) and reduced tillage (RT) influence soil physical, chemical, and biological indicators relevant to regenerative agriculture. Reduced or minimum tillage (RT/MT) and no-tillage (NT) reduce soil disturbance that restructures microbial habitats in prairie soil. Residue cover generate a buffered microenvironment by increasing soil moisture retention (+3.5-5.6% volumetric water content and balance temperature extremes which supports microbial survival, activity, and spatial continuity under the soil surface [35–37]. NT induces biologically mediated soil aggregation by fungal hyphae and microbial exopolysaccharides that resulted macroaggregate formation (7-38%) and reductions in wind-erodible fine aggregates [38,39]. Although initially increasing surface bulk density causes penetration resistance but these effects are depth limited and faded away with time as microbes accumulate organic matter and improve pore connectivity [40,41]. Together reducing tillage improves soil physical attributes that establish stable microbial habitats and trigger microbiome regeneration.

NT/RT enhance soil fertility primarily through robust microbe mediated carbon and nutrient transformations. Studies demonstrated that long term NT adoption resulted increased soil organic carbon 2.14 g kg<sup>-1</sup> in surface layers and approximately 4 Mg C ha<sup>-1</sup> over decades through stabilized recalcitrant carbon pools [42]. Moreover, residue retention and diversified crop rotations along with NT, enhance crop nitrogen uptake (13-47%) which indicate improved microbial mineralization and

nitrogen retention within the soil-plant system [38,43]. Long-term NT alleviate pH stratification, therefore underscore the transition from mechanical nutrient input to microbe mediate enhanced soil chemistry [44]. These positive responses in chemical indicators assures that NT promotes effective nutrient cycling by microbes rather than physical mixing.

Biological indicators provide the direct evidence of NT acting as a catalyst for microbial functional enhancement. Increasing microbial biomass carbon up to 86% in surface soils (0-5 cm), accompanied by elevated activities of key extracellular enzymes involved in carbon, nitrogen, and phosphorus cycling, including  $\beta$ -glucosidase, cellulase, xylanase, and phosphatase, were reported in 25-year-long Prairie experiments [45–48]. Compared to conventional tillage NT resulted 32-60% higher arbuscular mycorrhizal fungal (AMF) biomass, therefore preserving AMF network reinforce symbiotic nutrient acquisition and biologically driven aggregation processes [10,46]. Abundant functional genes including *nifH* and *nirK* in soil indicate enhanced microbial potential for nitrogen fixation and denitrification, although a few case studies also reported NT altered (*nirK* + *nirS*)/*nosZ* ratios indicating context-dependent trade-offs related to N<sub>2</sub>O emissions under specific moisture regimes [49]. Therefore, NT improves both microbial biomass and metabolic capacity, and pushing microorganisms as active agents for soil regeneration.

These integrated physical, chemical, and biological responses highlights NT/RT as a microbiome-centric mechanism for RA in Western Canada. By enhancing microbial stability, functional redundancy, and biogeochemical efficiency, NT build resilience to drought and climatic variability while maintaining productivity in Prairie agroecosystems [10,50]. Taken together, the studies in Table 3.1 show that NT/RT systems consistently enhance microbial biomass and activity, improve aggregation and SOC stabilization, and thereby position reduced disturbance as a core microbiome mediated mechanism for RA in Western Canada.

**Table 3.1.** Effects of no-till (NT) and reduced tillage (RT) on soil health indicators in Western Canada.

Soil health domain	Indicator	Effect of NT/RT relative to CT	Experimental design	Location of study	Findings	References
Physical	Soil moisture retention	+3.5-5.6% VWC; +0.3±0.02 m <sup>-3</sup> VWC	Long-term NT; residue retained; some NT + cover crop systems; Crop rotation	Saskatchewan n Alberta	Residue reduces evaporation; improved pore continuity and snow trapping	[35,36]
	Soil temperature	-0.8 to -9.9 °C (summer); warmer in winter +7-38%	Residue-covered NT soils; surface measurements	Ontario* Prairies	Residue buffers diurnal and seasonal thermal extremes	[35]
	Aggregate stability	macroaggregates (>12.7 mm); fewer fine aggregates	straw retained vs removed; multi-year trials	Saskatchewan n	Fungal hyphae and microbial binding agents preserved	[38,39]
	Bulk density	Slightly higher at surface (0-10 cm); structure improves over time	Long-term NT (0-15 cm); corn-soy and cereal systems	Ontario* Prairies	Reduced disturbance increases surface packing; SOC offsets compaction	[40,41]
Chemical	Soil organic Carbon (SOC)	Across long-term NT studies over 11-47 yrs increased upto 15.6 kg C ha <sup>-1</sup> yr <sup>-1</sup>	NT with reduced summer fallow; continuous and diversified rotations	Saskatchewan n; Alberta	Slower residue decomposition; enhanced C stabilization	[51]

Soil health domain	Indicator	Effect of NT/RT relative to CT	Experimental design	Location of study	Findings	References
<b>Biological</b>	Nitrogen availability	+13-47% crop N uptake	NT vs CT; straw retained (S) vs removed (NS); 0-120 kg N ha <sup>-1</sup>	Alberta; Saskatchewan	Enhanced microbial mineralization and N retention	[43]
	Soil pH stratification	Lower pH at 10-30 cm	Long-term NT; minimal soil mixing	Québec; Ontario*	Reduced vertical redistribution of acidity	[44]
	Microbial biomass C	+40-86% (0-5 cm)	Long-term NT (>25 yr); Prairie soil zones	Prairie sites	Habitat stability and increased organic inputs	[45,46]
	Enzyme activity	↑ β-glucosidase, cellulase, xylanase, phosphatase	NT vs CT across multiple Prairie sites	Alberta Manitoba Saskatchewan	Accelerated C cycling and nutrient turnover	[48,52]
	AMF biomass	+32-60%	NT surface soils; residue retention	Saskatchewan	Preservation of mycorrhizal networks	[46]
Functional N cycling genes	↑ nifH, nirK; altered (nirK + nirS)/nosZ	NT surface soils; moisture-responsive systems	Prairie sites	Enhanced N cycling with potential N <sub>2</sub> O trade-offs	[49]	

Footnotes: CT = conventional tillage; NT = no-till; RT = reduced tillage; SOC = soil organic carbon; AMF = arbuscular mycorrhizal fungi; VWC = volumetric water content. Note: Most NT effects are strongest in surface soil layers (0-5 cm and 0-15 cm). While NT may increase surface bulk density or penetration resistance in early years, long-term adoption consistently improves aggregation, microbial biomass, and water retention, leading to net gains in soil function and resilience. \* Examples from outside Western Canada to provide broader Canadian context.

### 3.2. Effects of Crop Rotation on Soil Health Indicators

Table 3.2 compiles key Prairie experiments demonstrating how diversified crop rotations alter soil physical structure, nutrient status, and microbial communities compared with simpler rotations or monocultures. Crop rotation significantly shapes soil microbial communities, exert both structural and functional influence that supports long-term soil resilience through improving moisture and thermal regimes. Multi-crop rotation for 12 years along with no-till-integrated systems resulted 30-34% higher water retention [29] and 78% increased soil porosity [53]. Seasonal variation of soil temperature, classified by warmer winter and cooler spring soils, reflects the combined effects of residue carryover and canopy effect in crop rotation systems [54]. Studies reported that microbial binding agents and biologically mediated pore formation enhance soil structural dynamics under crop rotation, therefore increase aggregate stability up to 5-20% and plant-available water capacity up to 16-17% [53]. Diverse crop rotations have higher levels of nutrient cycling, soil organic matter, and increases in bioavailable nitrogen, potassium, and appear to have broader soil microbial metabolic capabilities, perhaps owing to a greater diversity of residue input types, compared to monoculture [29,55]. Cover crops to increase the absorption and utilization of N during periods that would otherwise be fallow, and subsequently release N by mineralization [56,57]. One widely practiced alternative to replenish N to soils is the incorporation of legumes in crop rotations. Legume roots can associate with N<sub>2</sub>-fixing bacteria which can convert atmospheric N<sub>2</sub> to bioavailable N [56]. Commonly used legumes in temperate crop production regions include soybean and alfalfa (*Medicago sativa*), which, in rotation, benefit the yields of subsequent crops such as canola, maize, and wheat [56]. Diversified rotations increased biomass inputs (+2710 kg dry matter ha<sup>-1</sup>), supply substrates that fuel microbial growth and organic matter formation [53]. Crop rotation reported to decrease soil pH 0.2-0.4 unit and increase cation exchange capacity, which reflect the cumulative

effect of organic residues and microbial metabolites on soil chemical buffering and nutrient retention [58].

**Table 3.2.** Effects of crop rotation on soil health indicators in Western Canada.

Crop Rotation Type	Soil Health Domain	Indicator	Effects	Experimenta 1 Design	Findings	Location of Study	References	
Multi-crop rotation	Physical	Soil porosity	+78%	4-yr rotation; 7 Prairie sites	Root diversity increases macro- and microporosity	Prairie sites	[53]	
Diversified rotation under no-till		Soil porosity	+35-75% (0-7.5 cm)	Diversified crops + NT	Microbial aggregation enhances pore structure	Lethbridge (AB); Swift Current and Scott (SK)	[53]	
Crop rotation (mixed annuals)		Soil temperature	+2.5-5.7 °C (winter); -1.8-2.4 °C (spring)	Seasonal measurements	Residue and canopy effects buffer temperature	Ontario*	[65]	
Diversified rotation		Aggregate stability	+5-20% (water-stable aggregates)	Surface soil; 4-yr study	Microbial binding agents stabilize aggregates	Prairie sites	[53]	
Diversified rotation		Soil moisture retention	+0.01-0.03 m <sup>3</sup> m <sup>-3</sup> PAW; +8% microporosity; +16-17% PAWC	3 of 7 sites; 0-10 cm	Microbial aggregation improves water storage	Lethbridge (AB); Swift Current and Scott (SK)	[53]	
3-4 crop rotation (canola-wheat-pea/barley)		Soil moisture retention	+30-34%	12-yr rotation	Diverse rooting systems enhance pore continuity	Lacombe (AB); Swift Current and Scott (SK)	[29]	
Soybean-based rotations (2-3 crops)		Bulk density / resistance	↓ 0.3-0.5 MPa resistance; ~1 g cm <sup>-3</sup> (p = 0.020)	Strongest under no-till	Improved structure offsets compaction	Ontario*	[66]	
Long vs short rotation vs perennial forage		Chemical	Nutrient availability and SOC	Significant effects on NO <sub>3</sub> -N (p = 0.03), PMN (p = 0.02), SOC (p = 0.01)	4-yr vs 2-yr vs PER	Rotational legacy regulates microbial N cycling	MB; SK; AB	[29,67]
Multi-crop rotation			Biomass C input	+2710 kg DM ha <sup>-1</sup>	4-yr rotation	Increased residue inputs fuel microbes	Prairie sites	[53]
Soybean-based rotations		Biological	Soil pH and CEC	pH ↓ 0.2-0.4; CEC ↑ ~2 meq 100 g <sup>-1</sup>	Crop sequence effects	Organic inputs modify exchange capacity	Ridgetown (ON)*	[58]
Soybean-based rotations	Available P and K		+7-10 mg kg <sup>-1</sup> (P); +19-30 mg kg <sup>-1</sup> (K)	Rotational cropping	Microbial solubilization and residue cycling	Ridgetown (ON)*	[58]	
Long-term rotation	Electrical conductivity		~2 dS m <sup>-1</sup> (0.5 m depth)	Harvest-time moisture 20-30%	Improved water use reduces salt accumulation	Bow Island (AB)	[68]	
3-4 crop rotation	Community composition	↑ diversity (~50%); <i>Serratia</i> (3.0%),	12-yr rotation	Root exudate diversity selects functional taxa	AB; SK	[29]		

Crop Rotation Type	Soil Health Domain	Indicator	Effects	Experimenta 1 Design	Findings	Location of Study	References
<b>Rotation systems (meta-analysis)</b> <b>2-crop rotation (corn-soybean)</b>	Microbial biomass	Pseudomonas (3.3%) MBC +13%; MBN +16%; fungal biomass +45%	76 studies; <600 mm MAP	Rotation expands microbial C and N pools Elevated microbial metabolic activity	Temperate and semi-arid	[59]	
						Soil respiration	2-5 kg C ha <sup>-1</sup> d <sup>-1</sup>

Footnotes: PAW = plant-available water; PAWC = plant-available water capacity; PMN = potentially mineralizable nitrogen; SOC = soil organic carbon; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MAP = mean annual precipitation. Note: Crop-rotation effects are strongest in surface soils (0-10 cm) and intensify with increasing rotation complexity and duration, particularly when combined with no-till management. \* Examples from outside Western Canada to provide broader Canadian context.

Long-term rotations increased overall microbial diversity by approximately 50%, enriched with functionally important genera such as *Serratia* and *Pseudomonas*, indicates selective recruitment driven by diversified root exudates and residue inputs [29]. Meta-analysis in rotation vs continuous cropping systems globally support these trends, showing increase of 13% microbial biomass carbon, 16% microbial biomass nitrogen, and 45% fungal biomass across temperate and semi-arid systems (<600 mm MAP) [59]. Elevated biological indicators such as soil respiration rates (2-5 kg C ha<sup>-1</sup> d<sup>-1</sup>) under crop rotation reflect enhanced microbial metabolic activity and turnover of organic substrates [58].

Rotating crops introduces variability in root traits, rhizodeposition patterns, and residue inputs, which collectively stimulate a broader spectrum of microbial taxa. Different crops secrete unique root exudates that fosters functional microbial diversity, allowing the proliferation of beneficial microbial guilds such as nitrogen-fixing bacteria during legume phases, lignocellulose-degrading fungi following high-biomass crops, and phosphorus-solubilizing bacteria in nutrient-demanding rotations [60–62]. The cumulative effect of alternating rooting depths across a rotation cycle enhances the availability of subsoil nutrients, and reduces nutrient leaching risks, improving nutrient-use efficiency and long-term fertility. Deep-rooted crops such as sunflower or safflower promote microbial colonization in subsoil layers [63]. In contrast, shallow-rooted crops like lentils or wheat activate microbial turnover closer to the surface [64]. As like crop diversity, crop sequence in the rotation system has enormous significance to impact soil microbial abundance and structure, including plant pathogens [33]. Crops left a “legacy effect” on the preceding years’ crop root exudate profile, that modify the chemical, physical, and biological properties of soil [33,56]. Thus, crop rotation promotes microbial resilience by continually resetting selective pressures and preventing functional stagnation of the soil microbiome. Overall, the evidence in Table 3.2 indicates that diverse rotations increase biomass inputs, water storage, nutrient buffering, and microbial diversity, making rotation design a primary lever for regenerating soil function and microbiome resilience in Prairie RA systems.

### 3.3. Cover Cropping

Table 3.3 summarizes Canadian and Prairie studies on cover crops, highlighting their effects on soil microclimate, structure, nutrient cycling, and microbial activity during non-cash-crop periods. Cover crops alter residue chemistry, root exudation, and soil microclimate, which collectively exert strong microbiome-mediated effects during the non-cash-crop window. Cover crops shape outcomes, with measurable changes in microbial biomass, enzyme activity, and community structure emerging

alongside improvements in soil physical conditions that stabilize microbial habitats. Legume cover crops increased soil temperature by 2.5-5.7 °C in winter and 0.1-3.0 °C in spring under southwestern Ontario conditions [69], while mixed legume covers (alfalfa-clover-hairy vetch) reduced bulk density by 3-4% in Québec, indicating a habitat shift toward greater pore-space connectivity [70]. Between the years 2011 and 2016, winter cover crop usage increased by almost 20% in Saskatchewan and 10% in Manitoba [71]. Species-level variability within legumes was evident in Saskatchewan where tilled pea/lentil/*Lathyrus* covers increased aggregate stability by 2.3-4.1%, and sweet clover increased it by 65% under both tilled and no-tilled by enhanced microbial binding (e.g., EPS) formation and fungal hyphal aggregate reinforcement [72]. Cover crops initiate microbial responses partly by improving the physical habitat template (temperature, density, water, aggregation) that governs microbial persistence and colonization.

Cover crops regulate microbial nutrient cycling by changing substrate supply, nutrient capture, and mineralization dynamics. In Prince Edward Island, brown mustard, buckwheat, phacelia, and their mixtures significantly ( $p \leq 0.05$ ) increased P, K, and Ca of 4.76%, 6.67%, and 5.51%, respectively, under a no-synthetic-fertilizer condition, this outcome consistent with enhanced microbial mineralization under diversified residue inputs [73]. In a multi-province comparison spanning Carman (Manitoba), Saskatoon and Redvers (Saskatchewan), and Lethbridge (Alberta), nitrate-N ( $p = 0.03$ ), potentially mineralizable N ( $p = 0.02$ ), and SOC ( $p = 0.01$ ) differed significantly among crop-phase contexts (long vs short rotation vs perennial forage), highlighting that cover-crop effects on microbial N availability can be conditional on rotation structure and phase interactions rather than acting in isolation [74]. These chemical indicators implies that cover crops rewire microbially mediated nutrient retention and turnover, but the magnitude and direction mostly depend on system context (rotation phase, fertilization history, and residue management).

Biological indicators showed that cover crops restructure microbial communities that enhance resilience but also introduce functional trade-offs. In Ontario, rye and radish-based systems increased microbial biomass by 20.7-37% and increased microbial biomass by 90 mg C g<sup>-1</sup> TOC, alongside shifts in alkaline phosphatase activity (-18% and -2.6%), which indicate a strong cover-crop control over microbial C allocation and P-cycling enzyme expression [75]. In Prince Edward Island, ITS and 16S profiling showed statistically significant increases in fungal and bacterial metrics (4.85% and 3.02%, both  $p = 0.01$ ) and enrichment of symbiotrophic fungi and nitrification-related bacterial groups under sorghum-sudangrass and buckwheat demonstrating that cover crop identity can steer microbial guilds linked to N cycling and symbiosis [76]. Similarly, in Ontario, crop sequencing comparing oat, rye, radish, and rye-radish mixtures, radish and rye exerted strong influence on fungal diversity, explaining 76.91% of variability, with community shifts involving Actinobacteria, Firmicutes, and Ascomycota and overall diversity patterns explaining 74.18%, a signal consistent with cover-crop-driven niche restructuring via contrasting residue chemistry and root exudate profiles [29]. Cover crops do not merely “increase microbes”; they selectively reassemble microbial guilds (symbiotrophs, nitrifiers, decomposers) and regulate enzyme-mediated functions, with statistically supported shifts depending on species and mixtures.

In coastal British Columbia, grass covers improved soil water content (winter wheat: 0.02-0.06 kg kg<sup>-1</sup>) and moderated seasonal temperature (clover: +3 °C warmer in fall and -4 °C cooler in spring), reflects likely buffer microbial activity across freeze-thaw and wet-dry transitions [70]. Mixed systems further amplified multifunctionality: barley-rye mixtures increased aggregate stability by 10-32%, and radish/rye mixtures increased SOC relative to no-cover controls, support that functional complementarity can broaden microbial metabolic niches and enhance C stabilization [55,70,75]. Interestingly, cover crops integrated with grazing (corn/grazing-soybean/grazing-cover crop/grazing) increased soil organic matter by 20-26% and improved macro porosity, infiltration rate, and total porosity over the long term, indicating that coupling plant inputs with animal-derived substrates may intensify microbial turnover and aggregation pathways [79]. In summary, as synthesized in Table 3.3, cover crops tend to improve habitat conditions and microbially mediated nutrient cycling, but their magnitude and direction of effect depend strongly on species choice,

rotation phase, and climatic context, underscoring the need for context-specific RA design in the prairies.

**Table 3.3** Effects of cover crop functional groups on soil health indicators in Canada.

Cover crop functional group	Soil health domain	Indicator	Effect	Experimental design	Location of study	References
Legume cover crops (e.g., clover, alfalfa, vetch, sweet clover)	Physical	Soil temperature	+2.5-5.7 °C (winter); +0.1-3.0 °C (spring) at 15 cm	Legume cover crops under seasonal cover	Southwestern Ontario*	[69]
		Bulk density	3-4% decrease	Mixed legume covers (alfalfa-clover-hairy vetch)	Québec*	[70]
	Biological	Aggregate stability Microbial diversity	+2.3-4.1%; up to +65% (sweet clover) ↑ symbiotrophic fungi; ↑ nitrification-related bacteria	Tilled and no-till comparisons ITS and 16S amplicon sequencing	Saskatchewan Prince Edward Island*	[70] [76]
Grass cover crops (e.g., rye, barley, oat, winter wheat)	Physical	Bulk density	37-62% decrease (rye, sweet clover comparison)	No-till and rototill systems	Ontario*; Saskatchewan	[70]
		Soil temperature	+3 °C warmer (fall); -4 °C cooler (spring)	Seasonal monitoring	Eastern Canadian* Prairies	[70]
	Chemical	Soil water content	+0.02-0.06 kg kg <sup>-1</sup>	Winter wheat cover	Westham Island, BC*	[77]
		Soil organic C	+41% SOC	Rye cover under tillage	Ontario*	[70]
		Biological	Fungal and bacterial diversity	74-77% explained variability; ↑ Actinobacteria, Firmicutes, Ascomycota	Amplicon sequencing	Ontario*
Brassica cover crops (e.g., oilseed radish, mustard)	Chemical	Nutrient availability	P, K, Ca ↑ by 4.76-6.67% (p ≤ 0.05)	No synthetic fertilizer	Prince Edward Island*	[73]
		Nitrate-N and PMN	Significant effects (p ≤ 0.03)	Rotation and cover crop phases	MB; SK; AB	[74]
	Biological	Microbial biomass and enzymes	↑ 90 mg C g <sup>-1</sup> TOC; ↑ alkaline phosphatase	RAD and RAD+rye systems	Ontario*	[75]
Mixed cover crops (legume + grass / grass + brassica)	Physical	Aggregate stability	+10-32%	Barley-rye mixtures	British Columbia*	[70]
	Chemical	SOC and nutrient pools	SOC: No CC = 19.34; Rye = 26.01; RAD = 27.19; RAD+Rye = 26.42	Mixed covers	Prairie sites	[75]
	Biological	Microbial biomass and diversity	Microbial biomass ↑20.7-37%	Mixed covers	Ontario*	[75]

Cover crop functional group	Soil health domain	Indicator	Effect	Experimental design	Location of study	References
Cover crops with grazing	Physical and Chemical	SOM, porosity, infiltration	SOM ↑20-26%; ↑ macroporosity and infiltration	Integrated crop-livestock systems	Prairie sites	[79]

Footnotes: Abbreviations: SOC = soil organic carbon; SOM = soil organic matter; PMN = potentially mineralizable nitrogen; PAW = plant-available water; AMF = arbuscular mycorrhizal fungi; RAD = oilseed radish. Experimental context: Unless otherwise specified, reported responses were measured in surface soils (0-15 cm). Observed effects occurred under both tilled and no-till systems, with tillage interactions influencing the magnitude but not the direction of cover-crop responses. Methodological notes: Microbial diversity and community shifts were assessed using ITS and 16S rRNA amplicon sequencing. Reported diversity values represent treatment-explained variance from multivariate analyses rather than absolute richness. Interpretive qualifier: Several chemical and biological responses reflect interactive effects between cover crops and crop rotation phase or residue management, rather than cover crops acting in isolation. \* Examples from outside Western Canada to provide broader Canadian context.

### 3.4. Effects of Organic Amendments on Microbial Dynamics and Soil Health

The directional patterns synthesized and summarized in Table S 3.4 indicate that organic amendments consistently shift terrestrial ecosystem toward microbe regulated carbon and nutrient cycling, although the magnitude of response are strongly dependent on amendment quality and decomposability. Nutrient-rich organic inputs, especially livestock and green manures, produced statistically significant increases in microbial biomass and enzyme activity, which reflects rapid microbial growth fueled by labile carbon and nitrogen substrates [80]. In contrary, mineral-only fertilization either suppressed or failed to sustain microbial biomass, highlighting a fundamental disadvantage of chemically driven fertility system [80]. The strong positive indications in Table S 3.4a for microbial biomass and enzymatic activity under organic inputs represents accelerated microbial turnover and enhanced biochemical capacity, which altogether underpin improved soil organic matter formation and nutrient retention.

Distinct microbial strategies and emergent soil functions were found by using different types of organic amendment. manure and compost significantly increase microbial biomass, diversity, and soil organic carbon, which translates into robust yield responses, particularly in manure-amended systems where yield gains were highest. A meta-analysis across global systems found average yield increases of approximately 27% and up to 49% under manure amendments; similar patterns of yield and disease benefits are reported in Prairie studies and biorational management work. [80]. On the other hand, crop residues primarily stimulated carbon-degrading enzymes and microbial biomass but the outcome was associated with transient nitrogen immobilization, resulted weaker or delayed yield responses. While biochar can enhance stable SOC pools and microbial habitat structure, but the effects on nutrient cycling and yield were variable unless combined with nutrient-rich amendments, which underscore its capacity as a microbial habitat modifier rather than a central nutrient source [81]. These findings demonstrate that organic amendments do not uniformly increase microbes but instead reprogram microbial allocation strategies toward growth, niche stabilization, and enzyme production, according to substrate chemistry.

Organic amendments drive soil health improvements primarily through microbial emergence rather than direct nutrient supplementation in soil. Statistically significant gains in microbial biomass (up to 51%), enzyme activity, and carbon stabilization under organic inputs reflects improved nutrient-use efficiency and sustained yield benefits (up to 27%), particularly in low-SOC, semi-arid Prairie soils [80,82]. These outcomes (Table S 3.4b) reinforce that the RA practices improve soil dynamics by activating microbial processes that integrate carbon inputs, nutrient cycling, and soil structure into a resilient functional system.

### 3.5. Impact of Regenerative Agriculture (RA) Practices on Crop Yield and Long-Term Sustainability in Western Canada

Table 3.5 integrates yield and input-use outcomes from long-term RA trials across the Prairies and related temperate regions, linking the soil and microbial changes documented in Tables 3.1-3.3 to agronomic performance. In Western Canada, no-till and reduced-till systems substantially increase wheat yield (10% to 147%) range across multiple medium- to long-term experiments (4 to 10 years), under continuous wheat or rotation-integrated systems, larger gains occurred in severe drought years [50]. It is noteworthy that synthesis studies reported annual yield gains up to 10% across multiple crops (wheat, canola, pulses) under long-term no-till adoption, which suggest that productivity benefits accrue progressively as soil structure, organic carbon, and biological functional turnover [83]. In contrary, short-term or site-specific studies showed more variable responses, highlighting that time scale is a crucial factor to bring the agronomic benefits of RA, particularly under high interannual climate variability [44,84].

Crop rotation emerged as one of the most reliable RA strategies for simultaneous yield improvement and sustainability, especially when pulses were incorporated. Long-term rotations ( $\geq 12$ -30 years) consistently increased grain yield (14-38%) and protein yield (up to 66%) compared to continuous wheat, and benefits are most pronounced under semi-arid and low-fertility conditions, by improving biological nitrogen fixation and nutrient cycling, reducing system dependence on external inputs [55]. A multi-site Prairie studies also demonstrated moderate but consistent yield increases (0.1-0.5 t ha<sup>-1</sup>) of cereals through diversified rotations, even during dry years, indicating a buffering effect against climatic stress rather than yield maximization alone [53]. These findings indicate that diversified crop rotations enhance soil microbial biomass, functional diversity, and nitrogen-use efficiency, translating into more resilient yield performance across variable seasons [29,59]. The context-dependent yield outcomes by adopting cover crops and organic amendments reinforce the need to evaluate RA practices within specific environmental and management frameworks. Occasionally cover crops resulted small yield penalties during dry years or under suboptimal termination timing in semi-arid Saskatchewan [57]. In contrast, long-term experiments in Ontario demonstrated substantial cumulative yield gains in grain corn (38-59 bu ac<sup>-1</sup> over 14 years) under diverse legume and non-legume cover crop mixtures, particularly during mid-season droughts, highlighted the importance of temporal scale and moisture regime (GFO, 2007-2021). 25-year long stewardship program in southwestern British Columbia found up to 71% increase in soil mass SOC stocks and 25% higher soil workability threshold, indicating significant improvement agroecosystem resilience [84]. Comparative examples from humid temperate agroecosystems in Ontario and British Columbia provided complementary insights into long-term regenerative agriculture outcomes in broader Canadian contextual benchmarks. Organic amendments often delivered substantial yield benefits, with increase up to 49% reported in some systems under specific conditions, although responses vary with amendment type, application rate, soil condition and climate [80,81]. Together, the studies in Table 3.5 suggest that when RA practices are stacked and sustained, they tend to stabilize yields and reduce fertilizer and pesticide requirements, with soil microbial regeneration emerging as the common pathway connecting management changes to agronomic resilience in Prairie systems.

**Table 3.5** The impact of Regenerative Agriculture (RA) practices on Crop yield and sustainability indicators in Western Canada.

RA practices	Crop System	Yield Effect	Duration	Location	Experimental condition	Climate stress context	Sustainability signal	Key References
No till/ Reduced till	Wheat	+10-147%	1-10 yr	AB, SK (multiple sites)	Continuous wheat or combined with crop rotation (CR)	Drought-prone Prairies	Yield stability ↑; SOC accumulation	[50]

RA practices	Crop System	Yield Effect	Duration	Location	Experimental condition	Climate stress context	Sustainability signal	Key References
<b>Crop rotation</b>	Wheat (multi crop synthesis)	+7% yr <sup>-1</sup>	Long-term	Western and Eastern Canada*	NT adoption across cropping systems	Drought	Climate resilience ↑	[85]
	Canola	+10% yr <sup>-1</sup>	Long-term	Canada	NT systems	Variable Rainfall	Input efficiency ↑	[85]
	Pulses	+9% yr <sup>-1</sup>	Long-term	Canada	NT systems	Semi-arid	N-use efficiency ↑	[85]
	Mixed cereals	↔ /slight ↑	24 yr	Québec*	Long-term NT vs CT	Climate variability	Yield resilience ↑	[44]
	Canola-wheat-pea/barley	+421kg ha <sup>-1</sup>	12 yr	AB, SK	Diversified rotation	Variable precipitation	Stability ↑	[29]
	Wheat and cereals	+0.1-0.5 t ha <sup>-1</sup>	4 yr	AB, SK	Diversified rotation	Prairie drought	Yield buffering	[53]
	Wheat-canola-wheat-pea	+14-38%	>30 yr	SK	Long-term rotation	Semi-arid	NUE ↑; SOC ↑	[55]
<b>Cover crops</b>	Wheat-canola-wheat-pea	Grain +38%; protein +66%	12 yr	SK	Pulse inclusion	Drought	Nutritional resilience ↑	[55]
	Mixed cash crops	Slight ↓ (dry yrs)	1-3 yr	SK	Legume and grass covers	Low rainfall	Risk-reward trade-off	[57]
	Grain corn	+38-59 bu ac <sup>-1</sup>	14 yr	ON*	Legume and non-legume mixes	Mid-season drought	Long-term gain ↑	Experiment by Grain Farmers of Ontario (GFO) (2007-2021)
	Soybean-wheat-corn (organic)	Soybean +5-10%; wheat 8-9%	2-3 yr	ON*	Legume covers; organic	Variable seasons	System resilience ↑	Experiment by Grain Farmers of Ontario (GFO) (2022)
	Wheat, barley	+27-49%	Multi yr	AB, SK	Manure, compost, biochar	Semi-arid	Biological fertility ↑	[80,81].

Footnotes: \*Yield response expressed relative to conventional or control systems unless stated otherwise. ↔ indicates no statistically significant difference. SOC = soil organic carbon; NUE = nitrogen-use efficiency; NT = no-till; CT = conventional tillage; CR = crop rotation. Climate stress contexts are derived from site history (Prairie drought frequency, rainfall variability) reported in source studies. \* Examples from outside Western Canada to provide broader Canadian context.

## 4. Microbial Contributions to Control Prairie Pest and Reduce Synthetic Inputs

### 4.1. Effects of Regenerative Agriculture Practices on Weed, Insect, and Pathogen Suppression

Table 4.1 summarizes key field studies from Western Canada that examine how regenerative agriculture practices influence weed pressure, insect pests, and soilborne diseases through microbial and habitat-mediated mechanisms. Multiple long-term field studies across Alberta, Saskatchewan, and Manitoba indicate that regenerative agriculture practices have consistently reduced seedling emergence issues and soil-borne disease pressure at research sites throughout Western Canada. These suppressions of weeds and pathogens occurs primarily through microbial niche competition, antagonism, and soil network stabilization, with effectiveness scaling with time, diversity, and system complexity. A 4-year cereal-pulse-cover crop rotation at Lethbridge, Alberta, significantly

reduced germination of *Kochia scoparia* and *Amaranthus retroflexus* while suppressing root rot pathogens (*Fusarium graminearum*, *Rhizoctonia solani*) [86]. These effects were mechanistically linked to residue-driven allelopathic compounds and enhanced populations of antagonistic rhizobacteria (*Pseudomonas*, *Bacillus*), and arbuscular mycorrhizal fungi (AMF) colonization. Importantly, suppression occurred under rotation-based systems rather than cover crops alone, highlighting that microbial-mediated weed and pathogen control emerges strongly under multi-year, diversified systems, not under short-term interventions. Microbial-mediated mechanisms for suppressing pathogens, pests, and weeds are summarized in Table S 4.1.

Long-term no-till systems evidenced cumulative and stabilizing suppression effects. In a 12-year zero-till wheat-canola-pulse system at Swift Current, Saskatchewan, annual weed emergence (*Avena fatua*, *Setaria viridis*) declined over time, coinciding with reduced soil inoculum of *Fusarium* spp. and *Gaeumannomyces graminis* [87]. These outcomes were associated with preserved fungal hyphal networks, increased actinomycetes abundance, and enrichment of weed-suppressive *Pseudomonas fluorescens*. Notably, suppression strengthened with duration, underscoring that soil microbial network stabilization, rather than immediate disturbance reduction. A 6-year high-residue wheat-canola rotation in Manitoba delayed spring weed emergence and reduced early-season foliar diseases via saprophytic fungi (*Streptomyces*) and phenolic compound release during residue decomposition [88]. These results demonstrate that residue quantity and persistence are critical moderators of microbial antagonism.

Integrated systems intensified suppression through trophic and microbial feedbacks. In Carman, Manitoba, a 3-year cover crop-livestock integration reduced perennial weed regrowth (*Cirsium arvense*) and decreased clubroot severity (*Plasmodiophora brassicae*) in canola rotations [89]. Manure inputs introduced rumen-derived microbial consortia, enhanced soil microbial diversity, and accelerated decomposition-driven allelochemical release, illustrating how livestock integration strengthens microbial competition and induced resistance. An 8-year high-diversity rotation at Lacombe, Alberta significantly reduced root disease buildup (*Fusarium avenaceum*, *Pythium* spp.) through diversified root exudation and increased AMF colonization [90]. Collectively, these studies suggest that pathogen and weed suppression under RA often emerges from greater microbial diversity, functional redundancy, and network stability, and that effectiveness depends on rotation length, residue continuity, and system integration rather than any single practice. Taken together, the experiments in Table 4.1 show that long-term, diversified RA systems can reduce weed emergence and pathogen pressure primarily by enriching antagonistic and competitive microbial guilds, so that durable pest suppression in Prairie agroecosystems emerges from restored soil microbial networks rather than any single practice, and these same microbe-mediated controls reduce dependence on synthetic inputs while reinforcing soil health and yield resilience, linking suppression outcomes (Table 4.1) to the microbial-driven soil processes (Tables 3.1-3.3) and the yield responses summarized in Table 3.5.

**Table 4.1** Effects of Regenerative Agriculture practices on weed, insect, and pathogen suppression in Western Canada.

Regenerative practice	Weed suppression (directional)	Insect / disease suppression (directional)	Key target species	Dominant microbial mechanisms	Experimental location and Reference duration
Cover crops	↓ weed emergence	↓ root rot incidence	<i>Kochia scoparia</i> , <i>Amaranthus retroflexus</i> ; <i>Fusarium graminearum</i> , <i>Rhizoctonia solani</i>	Allelopathic phenolics; <i>Pseudomonas</i> , <i>Bacillus</i> antibiotics; AMF-mediated nutrient competition	Lethbridge, AB; 4-yr cereal-pulse-cover crop rotation [86]
No-till / minimal disturbance	↓ annual weeds (long-term)	↓ soilborne pathogens	<i>Avena fatua</i> , <i>Setaria viridis</i> ; <i>Fusarium</i> spp.,	Preserved AMF hyphal networks; antagonistic actinomycetes; weed-	Swift Current, SK; 12-yr zero-till [87]

<b>Residue retention</b>	Delayed emergence	↓ early-season leaf disease	<i>Gaeumannomyces graminis</i> , <i>Kochia scoparia</i> , <i>Chenopodium album</i> ; <i>Alternaria brassicae</i> , <i>Leptosphaeria maculans</i>	suppressive <i>Pseudomonas fluorescens</i> ; Saprophytic fungi; <b>Streptomyces</b> ; antifungal phenolics; N immobilization	wheat-canola-pulse system Brandon, MB; 6-yr high-residue wheat-canola rotation	[88]
<b>Cover crops + grazing</b>	↓ perennial regrowth	↓ clubroot severity	<i>Cirsium arvense</i> ; <i>Plasmodiophora brassicae</i>	Manure-borne microbes; fungal decomposition; induced systemic resistance	Carman, MB; 3-yr crop-livestock integration	[89]
<b>High-diversity rotations</b>	-	↓ root disease buildup	<i>Fusarium avenaceum</i> , <i>Pythium</i> spp.	Diverse root exudates; AMF colonization; microbial niche competition	Lacombe, AB; 8-yr diversified rotation	[90]

Footnotes: ↓ = statistically significant reduction relative to conventional practice. AMF = arbuscular mycorrhizal fungi. Suppression effects are context-dependent and strongest under diversified rotations and sustained residue inputs. Insect suppression is primarily indirect, mediated through habitat complexity, microbial antagonism, and improved crop vigor rather than direct toxicity.

#### 4.2. Microbial Contributions to Input Reduction and Agronomic Efficiency

Table 4.2 compiles studies and decision-support frameworks that quantify how microbially mediated processes under RA such as biological nitrogen fixation, residual N carryover, and improved pest regulation, translate into reduced fertilizer and pesticide inputs without sacrificing yield. In Western Canada, agrochemicals are extensively used to support the productivity of major crops by improving crop nutrition, suppressing weeds, controlling pests, and plant diseases. Herbicides are the most widely applied chemicals in this region, with active ingredients like glyphosate, 2,4-D, and dicamba commonly used to manage weeds and facilitate conservation tillage systems [91]. Fungicides such as azoxystrobin and propiconazole are used to control diseases such as sclerotinia, fusarium head blight, and rusts in crops like canola and wheat [92,93]. Judicious selection of fungicides and optimal application timings and methods, can enhance wheat yield stability and quality in environments with a high risk of Fusarium head blight (FHB) (*Fusarium* spp.) [94]. Insecticides such as lambda-cyhalothrin and deltamethrin are used to manage pests like flea beetles and cutworms, particularly in canola [95]. Additionally, synthetic fertilizers, primarily nitrogen (e.g., urea, anhydrous ammonia), phosphorus (MAP), potassium, and sulfur, are essential inputs for crop nutrition and yield optimization [96].

Across Western Canadian agroecosystems, RA practices consistently reduce dependence on synthetic nitrogen and chemical pesticides, but the magnitude and reliability of these reductions are strongly conditioned by practice integration, duration, and crop context (Table 4.2). Long-term adoption of no/reduced-till systems combined with diversified crop rotations in prairie croplands resulted reduced need for synthetic N (up to 73%), herbicide (up to 42%) application [97–99]. These reductions were not attributable to single-year fertilizer inputs rather emerged from soil organic matter (SOM) accumulation, enhanced nitrogen cycling, and improved disease suppression, particularly under integrated weed management frameworks [100]. The long temporal scale of these experiments also highlights that RA-driven input reductions are system-level outcomes, rather than immediate substitutions for conventional inputs.

Microbial nitrogen fixation was most pronounced in systems which incorporate pulse intercropping. Residual N follow pulse phases ranged from 10-60 kg N·ha<sup>-1</sup>, with conservative recommendations of 10-20 kg N·ha<sup>-1</sup> under Prairie risk-management frameworks [47,101]. Intercropping (e.g., pea-oat, pea-canola) further reduced mineral N demand by 5-25 kg N·ha<sup>-1</sup> while simultaneously lowering fungicide and insecticide use across sites in Alberta, Saskatchewan, and Manitoba [102]. Rhizobial inoculation of pulses replaced tens of kilograms of synthetic N per hectare

through biological fixation, reducing fertilizer dependency by up to 30%, although benefits were variable across soils and seasons [103,104]. These findings underscore the microbial symbioses for sustainable N-use efficiency, rather than only fertilizer use reductions. Overall, the evidence synthesized in Table 4.2 indicates that as RA practices rebuild microbial nutrient cycling and biotic pest control, Prairie producers can lower synthetic N, herbicide, and insecticide use while maintaining or improving yield stability, reinforcing the view that input reduction is a downstream outcome of functional soil microbiomes rather than a stand-alone management target. RA-driven reductions in chemical inputs are emergent properties of biologically functional systems, where microbial regulation, crop diversity, and adaptive management combinedly lower external input requirements while sustaining productivity and environmental performance under Prairie conditions.

**Table 4.2** Effects of regenerative agriculture on reduction of synthetic inputs and sustainability outcomes in Western Canada.

Regenerative practice	Synthetic N reduction† (kg N·ha <sup>-1</sup> )	Reduction in use of Herbicide, Fungicide and Insecticide	Primary sustainability benefits	Representative locations	References
No-/reduced till + diversified rotation	Gradual; cumulative (long-term)	Variable; ↓ under IWM	Soil conservation; SOC gain; reduced runoff and fuel/GHG emissions	Lacombe AB; Swift Current and Scott SK	[47,99]
Cover crops (short mixes / overwintering)	0-15	20% reduction in disease cycle reduce up to 15% foliar disease pressure indirectly	N retention; soil structure; beneficial insect habitat	SK; AB;	[105,106]
Crop rotation (incl. pulses)	10-60‡ (conservative: 10-20)	Variable Reduce pest pressure	Improved NUE; lower N <sub>2</sub> O intensity	Brooks AB	[47]
Intercropping (e.g., pea-oat, pea-canola)	5-15	5~30% lower disease spread	Whole-system resilience; yield stability	Lacombe and Lethbridge AB; Melfort SK; Brandon MB	[102,107]
Rhizobial inoculation (pulses)	Tens (variable; fixation-driven)	Indirect; minor effect	Substitutes synthetic N (≤30%)	Lethbridge AB; Swift Current and Canora SK	[47]
Integrated weed management (IWM) and HWSC	N/A	Indirect; system specific	Long-term weed seedbank depletion	Lethbridge AB; Swift Current and Canora SK	[108]
Split and precision N management	10-30	Variable, reduced volume via targeted spraying	Reduced N losses and N <sub>2</sub> O emissions	Indian Head SK; PEI	[109,110]
Cultivar selection and economic thresholds (IPM)	N/A	10-50 (resistant cultivars + thresholds)	Biodiversity protection; beneficial insects and microbes	Prairie-dominant, multi-site Canada	[111,112]

Footnotes: † Synthetic N reduction refers to avoided fertilizer inputs relative to conventional management, not total crop N demand. ‡ Residual N credits are highest following pulse phases; conservative values reflect risk-averse recommendations under Prairie conditions. IWM = integrated weed management; HWSC = harvest weed seed control; NUE = nitrogen use efficiency. Reductions are context-dependent (crop, soil, climate, pest pressure) and generally strongest in multi-year systems.

## 5. Discussion

### 5.1. *Microbial Resuscitation as the Integrating Mechanism of Regenerative Agriculture*

Figure 1 conceptualizes regenerative agriculture (RA) as a process that rebuilds soil systems primarily through biologically mediated mechanisms rather than through simple substitution of conventional inputs. Decades of intensive tillage, monocropping, summer fallow, and heavy agrochemical use in the Canadian Prairies have progressively damaged microbial habitats, disrupted fungal networks, and narrowed functional genetic diversity, contributing to poor soil structure, inefficient nutrient cycling, and inconsistent crop performance. Evidence synthesized across this review and in comparable global assessments (76, 109, 110) indicates that RA practices such as no tillage, diversified rotations, cover cropping, organic amendments, and crop-livestock integration can improve microbial habitat continuity, increase carbon inputs to the rhizosphere, and enhance functional redundancy within soil microbial communities, although responses remain context dependent. As detailed in Tables 3.1-3.5, adopting these RA practices are associated with higher microbial biomass, greater enzyme activity, increased arbuscular mycorrhizal fungi (AMF) abundance, and elevated expression of genes involved in C, N, and P cycling, aligning with the mechanisms depicted in Figure 1. Intact hyphal networks and greater rhizodeposition support soil aggregate formation, water retention, soil organic carbon stabilization, and diverse microbial guilds strengthen biological regulation. At the same time, microbial recovery is neither instantaneous nor guaranteed. Systems with severely depleted SOC, compacted subsoils, or chronic moisture and temperature limitation may show muted or delayed responses, indicating that regenerative transitions require both sufficient time and minimum biophysical thresholds to be crossed before consistent functional gains are realized.

Taken together, RA outcomes in prairie systems can be viewed as a stimulation-response-outcome cascade, in which management changes first alter disturbance and resource regimes, then trigger microbial responses that reorganize soil function and associated ecosystem services. This synthesis therefore reframes regenerative agriculture not as a discrete set of practices, but as a management strategy aimed at restoring soil as a self-organizing biological system, with microbial network integrity acting as a central-though not exclusive-control point for soil process and agronomic outcomes.

### 5.2. *Microbial Restoration as the Foundation for Pest Suppression and Input Reduction*

Pest suppression and input reduction in regenerative systems are best viewed as emergent ecosystem properties of restored soil microbiomes, rather than as direct outcomes of any single agronomic intervention. The patterns of soil biological recovery summarized in Table 3.1-3.3 and Table S 3.4a-b provide a mechanistic insight for the consistent reductions in weeds, plant pathogens, and chemical inputs observed in the studies documented in Table 4.1-4.2. Across Prairie field studies, weed suppression, disease regulation, and lower pesticide dependence emerge not from any single management actions, but from the collective effects of microbial niche competition, antagonistic interactions, and stabilization of soil microbial networks. Long-term reduced tillage and diversified crop rotations selectively enrich populations of *Pseudomonas*, *Bacillus*, *Streptomyces*, and arbuscular mycorrhizal fungi (AMF), providing a plausible biological explanation for sustained suppression of *Fusarium*, *Rhizoctonia*, and weed establishment over time [90,112]. The inclusion of cover crops and organic amendments further amplifies enzyme activity and accelerates decomposition of allelopathic residues, reinforcing microbial dominance within the rhizosphere and strengthening biotic control of pests and pathogens [113]. Importantly, these microbe mediated processes translate into quantifiable reductions in synthetic inputs. As summarized in Table 4.2, residual nitrogen contributions from pulse-based crop rotations, symbiotic N fixation by rhizobia, and improved microbial nitrogen-use efficiency collectively reduce fertilizer requirements by approximately 10-60 kg N ha<sup>-1</sup>, while integrated weed management, cultivar selection, and threshold-based decision frameworks decrease herbicide and insecticide use by roughly 20-60% without compromising yield stability [47,102,114,115]. Together, these findings support the central premise of Figure 1: in prairie

agroecosystems, lower external input dependence emerges from biologically functional, microbial regulated soils, rather than serving as a primary starting condition for regenerative management.

### *5.3. Yield Stability and Prairie-Global Convergence Under Climatic Constraint*

The yield responses synthesized in Table 3.5 broadly mirror patterns reported in the global regenerative agriculture (RA) literature, but they are more tightly constrained by the distinctive climate of the Canadian prairies. Globally, RA is associated with consistent increases in soil organic carbon, microbial biomass, and ecosystem resilience, while yield outcomes remain context dependent, frequently neutral to positive in rainfed and biologically diversified systems, yet more variable where water or nutrient competition is poorly managed [116,117]. Prairie studies align closely with this global pattern: long-term no-till and diversified rotations reliably improve yield stability and drought resilience, whereas short-term yield penalties following cover crop adoption occasionally occur in short-season, moisture-limited condition [57]. Crucially, these trade-offs do not imply a failure of RA principles but instead reflects temporal and climatic modulation of microbial-mediated feedbacks. Yield responses therefore appear to follow a threshold pattern, in which early biological recovery improves resilience first, while consistent yield gains emerge only after cumulative improvements in aggregation, SOC stabilization, and nutrient buffering are achieved. Low soil temperature, episodic drought, and slower residue decomposition can delay the conversion of microbial recovery into yield gains. Figure 1 captures this dynamic by emphasizing time, practice stacking, and context specificity as prerequisites for interpreting agronomic outcomes. Where RA practices implemented as integrated, tailored to local climate, soil conditions, and production constraints, yield benefits tend to accumulate and interannual variability declines, consistent with long-term global findings [80].

### *5.4. System-Level Implications and Global Relevance of Prairie Evidence*

Synthesizing evidence from Prairie field experiments (Tables 3.1-3.5;4.1-4.2) and global meta-analyses demonstrated that the core biological mechanisms underpinning regenerative agriculture (RA) are broadly conserved across agroecosystems, including enhanced microbial biomass, restored fungal:bacterial balance, strengthened AMF networks, and improved carbon stabilization pathways. However, the magnitude, rate, and reliability of outcomes vary substantially, depending on alignment with local climate, soil texture, baseline soil organic carbon, and water regimes. Recent global syntheses consistently report that integrated RA systems restore microbial network stability, increase microbial carbon-use efficiency, and reinforce biologically mediated pest regulation, thereby enhancing yield resilience under climate stress [23,32]. Prairie datasets mirror these global patterns but further illustrate that soil health recovery precedes yield gains, particularly under semi-arid and short-season constrains. For example, long-term no till and diversified rotations in Saskatchewan and Alberta increased SOC stocks, microbial biomass, and enzyme activity (Tables 3.1-3.2), while yield stabilization became evident only after multi-year system restructuring (Table 3.5). Thus, prairie evidence confirms that regenerative transitions operate through cumulative microbial feedbacks rather than single practice effects, reinforcing the global conclusion that system integration, not isolated adoption, determines resilience trajectories.

Importantly, the principal distinction between global and Western Canadian RA outcomes lies not in the underlying microbial processes, which remain mechanistically similar, but in the temporal dynamics through which biological recovery translates into consistent yield and input-efficiency benefits under climatic constraint. In moisture-limited Prairie systems, low soil temperatures and episodic drought can delay residue decomposition, microbial turnover, and nutrient mineralization, slowing the agronomic expression of microbial gains. Yet once SOC thresholds and microbial network stability are achieved, Prairie systems exhibit pronounced improvements in drought buffering, disease suppression, and reduced synthetic N and pesticide reliance (Tables 4.1-4.2), aligning closely with global resilience patterns reported in semi-arid and temperate systems [80,85,117]. Taken together, these findings position the Canadian Prairies as a globally informative

stress-test for regenerative systems, a region where climatic limitation sharpens the signal of microbial functionality, thereby clarifying how carefully calibrated, multi-year regenerative strategies can sustain productivity, suppress pests, and reduce chemical dependence under environmental constraint.

Future research should prioritize long-term, systems-level experiments that explicitly quantify microbial network topology, carbon-use efficiency, and microbial necromass accumulation alongside crop performance metrics, while experimentally testing practice stacking, sequencing, and transition thresholds required to achieve functional stability in cold and water-limited soils. These initiatives will be essential for translating regenerative principles into regionally optimized management frameworks.

## 6. Conclusions

This synthesis supports the central hypothesis that regenerative agriculture (RA) enhances yield stability and long-term sustainability in the Canadian Prairies primarily through the restoration of soil microbial diversity, functional activity, and network resilience. Across long-term field evidence, regenerative management consistently strengthened microbial biomass, enzymatic capacity, mycorrhizal connectivity, and nutrient-use efficiency, alongside gains in soil organic carbon, aggregation, and water retention demonstrating that soil biological recovery underpins improvements in system performance. The hypothesis is strongly validated in relation to soil health, pest suppression, and reduced synthetic input reliance, and partially validated in terms of yield response, as agronomic gains tend to emerge progressively and remain sensitive to climatic and edaphic constraints. Although overall trends are positive, short-term and site-specific outcomes range from negative to positive, underscoring the importance of temporal scale in climate-variable Prairie systems. In semi-arid, short-season Prairie environments, microbial regeneration precedes consistent yield stabilization, indicating that resilience develops through cumulative ecological adjustment rather than immediate output increases. This pattern aligns with global regenerative systems while highlighting the Prairie region as a climate-constrained test case where biological processes are closely linked to risk reduction and sustainability outcomes. Viewed through this lens, RA represents not a set of discrete practices but a reorientation of management toward rebuilding soil as a living, adaptive system one in which microbes act as the primary drivers of ecosystem regulation and long-term agricultural resilience. Future research should advance integrated, long-term microbial monitoring frameworks to guide climate-adaptive regenerative transitions.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S 2.6 Core principles of regenerative agriculture (RA) mapped to microbial mechanisms and ecosystem outcomes. Table S 3.4a Effects of organic amendments on microbial indicators and soil health. Table S 3.4b Organic amendments and their microbiome-mediated impacts on soil health. Table S 4.1 Mechanism behind the Regenerative Practices to suppress Weed, Insect, and Pathogen.

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## Abbreviations

The following abbreviations are used in this manuscript:

RA	Regenerative Agriculture
SOC	Soil Organic Carbon
NT	No-Till
RT	Reduced Till
CT	Conventional Tillage
AMF	Arbuscular Mycorrhizal Fungi
VWC	Volumetric Water Content
PAW/PAWC	Plant-Available Water/ Plant-Available Water Capacity
PMN	Potentially Mineralizable Nitrogen
MBC/N	Microbial Biomass Carbon/Nitrogen
MAP	Mean Annual Precipitation
RAD	Oilseed Radish
NUE	Nitrogen-Use Efficiency
IWM	Integrated Weed Management
HWSC	Harvest Weed Seed Control

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