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VALIDATING SOIL METAGENOMICS FOR PRECISION AGRICULTURE: AN EVIDENCE-BASED FRAMEWORK FOR AGRONOMIC DECISION-MAKING

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ABSTRACT:
Soil metagenomics has recently developed as a valuable method for guiding precision agriculture. The attention to the use of microorganisms as indicators has increased for different purposes, such as managing fertilizers, suitability evaluation for certain crop cultures, and monitoring plant disease, there is a distinct deficit in standardized validation, confidently reporting, and governance of microbial indicators. The Validation Framework for Metagenomics-Driven Agronomic Recommendations (VMDAR) that provides a stepwise procedure through three phases with eight steps for progressing biologically defined microbial indicators toward feasible and practical agronomic recommendation tools. The framework incorporates a four-tier evidence hierarchy based on the GRADE method, a multidimensional confidence scoring system that evaluates Data Quality, Evidence Level, Seasonal Stability, and Spatial Validity, established statistical acceptance benchmarks, and governance supervision via a Scientific Advisory Board (SAB). The framework is demonstrated through four representative hypotheses concerning the sufficiency of sequencing depth, reproducibility of rhizosphere enrichment, reproducibility of indices, and the agronomic effectiveness of biologically informed recommendations. VMDAR integrates evidence assessment, confidence evaluation, validation, and governance into one cohesive framework. The proposed approach recognizes that biological observations alone are insufficient to support agronomic recommendations and therefore require structured evaluation before implementation. VMDAR helps ensure that its recommendations are founded on evidence and appropriate for evidence-based agricultural decision making and field application by linking metagenomic indicators with clearly established validation and decision-making protocols.

KEYWORDS: Soil Metagenomics; Precision Agriculture; Agronomic Recommendations; Evidence Hierarchy; Validation Framework; Soil Health

Abbreviations
CEGIF – Calibration-First Environmental Genomics Intelligence Framework
VMDAR – Validation Framework for Metagenomics-Driven Agronomic Recommendations
NAP – Nitrogen Availability Potential
PMP – Phosphorus Mobilization Potential
KCP – Potassium Cycling Potential
SBHS – Soil Biological Health Score
DPI – Disease Pressure Indicator
DIPS – District Investment Priority Score
STCR – Soil Test Crop Response
RCBD – Randomized Complete Block Design
SAB – Scientific Advisory Board
OC – Overall Confidence
DQ – Data Quality
EL – Evidence Level
SS – Seasonal Stability
SV – Spatial Validity
SOC – Soil Organic Carbon
MBC – Microbial Biomass Carbon
NUE – Nutrient Use Efficiency
PGPR – Plant Growth-Promoting Rhizobacteria
PSB – Phosphate Solubilizing Bacteria
KMB – Potassium Mobilizing Bacteria
NGS – Next-Generation Sequencing
LIMS – Laboratory Information Management System

BNF – Biological Nitrogen Fixation
RT-qPCR – Reverse Transcription Quantitative Polymerase Chain Reaction
VCR – Value-Cost Ratio
CBR – Cost-Benefit Ratio
RDF – Recommended Dose of Fertilizer
BLK – Bulk Soil
RZS – Root Zone Soil
RHZ – Rhizosphere Zone
MISH – Molecular Index of Soil Health
EMP – Earth Microbiome Project
ICAR – Indian Council of Agricultural Research
ICRISAT – International Crops Research Institute for the Semi-Arid Tropics

METHODOLOGY OF LITERATURE REVIEW:
Relevant literature was collected from scientific databases, including Google Scholar, PubMed, Scopus, and the Web of Science. Peer-reviewed research articles, review papers, methodological guidelines, and validation studies related to soil metagenomics, environmental genomics, microbial functional traits, soil health assessment, evidence-based decision-making, precision agriculture, and agronomic recommendation systems were examined. The reviewed literature provided the scientific basis for identifying current limitations in translating metagenomic observations into practical agricultural recommendations and informed the development of the Validation Framework for Metagenomics-Driven Agronomic Recommendations (VMDAR). Emphasis was placed on evidence of evaluation, validation requirements, reproducibility, quality assurance, confidence assessment, and governance mechanisms relevant to deployment-oriented metagenomic applications. The selection process used to identify, screen, evaluate, and include relevant studies for this review is summarized in Figure 1.

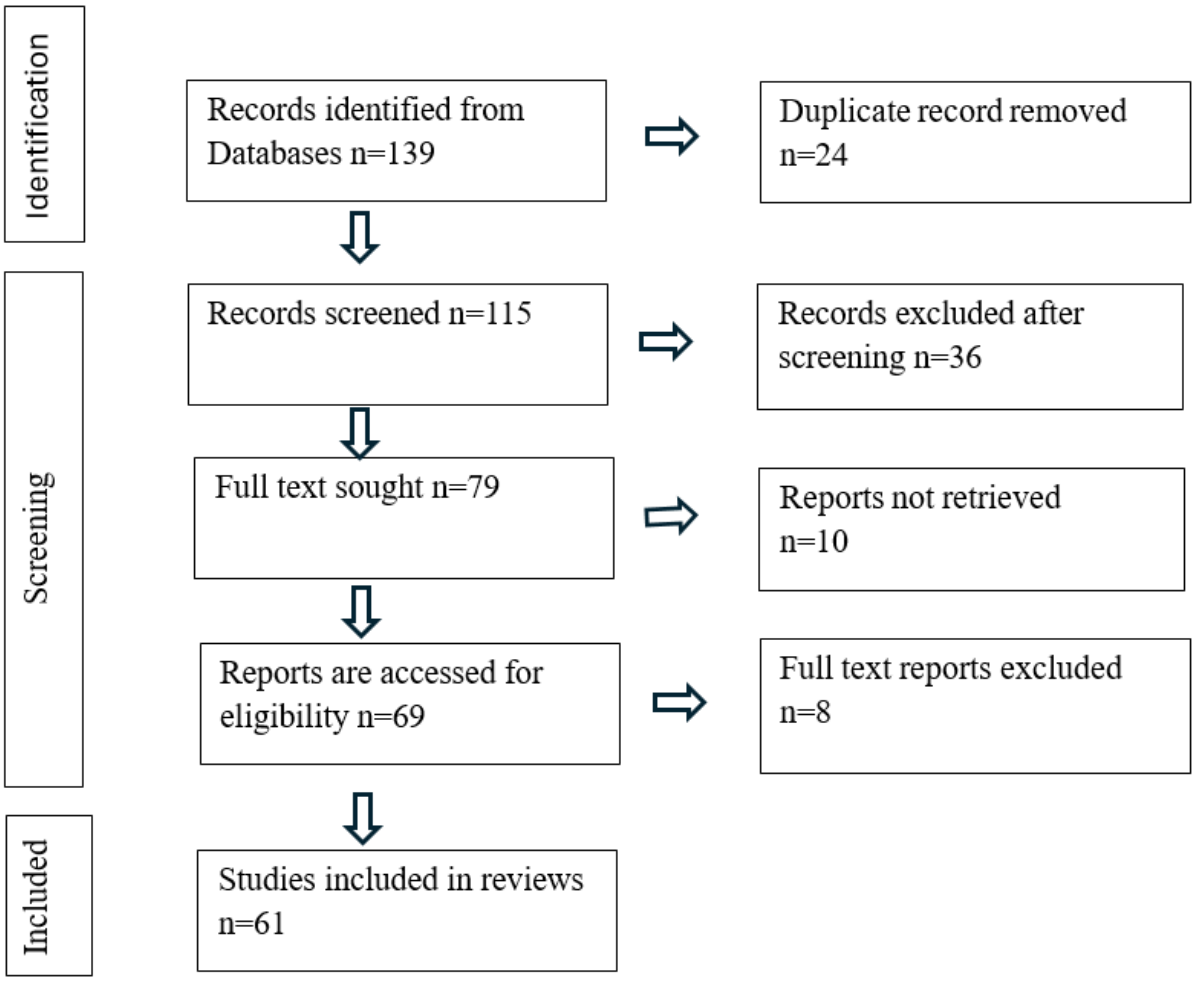

Figure 1. The PRISMA flow diagram illustrates the selection process used for identifying, screening, evaluating, and including studies considered in the development of the VMDAR framework:

1. INTRODUCTION:
In soil sciences, developments in metagenomics have recently provided a remarkable ability to characterize microbial diversity and functions within agro-ecosystems (Fierer, 2017; Thompson et al., 2017). For example, global studies, such as the Earth Microbiome Project and soil health assessment methods, are demonstrating the rise of environmental

genomics for understanding the cycling of nutrients and diseases or controlling production (Thompson et al., 2017; Deel et al., 2024). The number of studies demonstrating a correlation between microbial genes involved in function and relevant agronomic process is also rising (Prosser and Nicol, 2008; Louca et al., 2018). Although soil metagenomics has generated valuable insights into soil biological processes, their translation into routine agricultural decision-making remains limited. A biological indicator often shows a clear relationship with a soil process under one set of conditions but fails to demonstrate the same pattern elsewhere (Witt and Dawe, 2004; Vanlauwe et al., 2010). Such variability makes it difficult to decide when biological observations can be translated into dependable agronomic recommendations. Meanwhile, the rapid growth of metagenomic technologies has expanded the amount of information available on soil microbial communities and their functions (Fierer, 2017; Louca et al., 2018). To improve the practical use of these data, researchers have developed approaches ranging from functional gene profiling and metagenomic sequencing to soil health assessment frameworks, machine learning methods, and precision agriculture decision-support tools (Deel et al., 2024; Thompson et al., 2017). Although they hold scientific importance, most current frameworks emphasize biological characterization and indicator creation, with significantly less focus on evidence classification, validation needs, confidence evaluation, statistical acceptance standards, and governance of deployment.

2. Differentiating Biological Signals from Deployable Recommendations:

The increasing use of soil metagenomics in agriculture has highlighted the need to distinguish between biological observations and those that can support practical management decisions. Different kinds of outputs depend on distinct levels of evidence and therefore have differing degrees of scientific certainty. While some metagenomic results depend on established techniques and well-defined biological mechanisms, others require additional verification before being transformed into management recommendations. Consequently, the essential requirement of any deployment-oriented validation framework is to distinguish between outputs that are currently justifiable and those that rely on the generation of further evidence (Louca et al., 2018; Prosser and Nicol, 2008; Guyatt et al., 2011).

2.1. Biological Observations Supported by Current Evidence:

In the context of soil metagenomics, applications supported by established measurement methods generally provide the highest level of scientific confidence and scientifically defensible outcomes. Using metrics of microbial diversity, taxonomy, functional gene prevalence for soil health assessment has reliable results using approved analytical tools that provide good communication ability (Fierer, 2017; Nannipieri et al., 2012). In the same way, pathogens detection in Shotgun metagenomics (Mendes et al., 2011), the biological agents for effective disease control (Harman et al., 2004; Weller, 2007), Rhizobium-legume symbiont (Herridge et al., 2008), and the chemical element of ICAR-AICRP STCR framework were all derived from many experimental evidence and strong experimental confirmation (Murthy et al., 2024). Nevertheless, even though the biological processes are accurately described, their utility as reference and guidance to a specific management decision only depends on empirical adaptation and validation within specific condition, hence direct observation and confirmed method is strong reference, interpretations and consequence of interpretation still needs exploration (Guyatt et al., 2011).

2.2. Establishing the Evidence Needed for Agronomic Implementation:

The greatest degree of uncertainty is often found when biological observations indicate directions that are appropriate for agronomic interventions. It is possible to derive a significant insight into nutrient cycling and ecosystem function from measurements of functional gene number, biological indices and metagenomic features, yet the presence of biological signatures alone cannot be used to conclude that fertilization rates can be reduced without compromising crop yield (Prosser and Nicol, 2008; Louca et al., 2018). Justification of the Use of Such Observations in Management Recommendations only becomes possible when experimental tests are carried out in a variety of different environments, managements and soil types (Witt and Dawe, 2004; Vanlauwe et al., 2010; Piepho et al., 2011). It will take more than a single test to obtain the confidence required. It is important to calibrate regional effects, to gain independent verification and to continue tests under real farm conditions to identify where any relationship persists over time and space (Witt and Dawe, 2004; Vanlauwe et al., 2010).

3.Evidence Hierarchy For Metagenomics-Driven Recommendations

3.1. Rationale for an Evidence Hierarchy:

One aspect of interpreting biological observation in environmental genomics to date has been an lack of rigorous consideration for the evidence underpinning our interpretation. The issues described with the interpretation of the quality of evidence are not unique to the environmental genomics world and issues have long been raised in these fields: human medicine, human health, human policy, human research etc., where decision makers are faced with the choice between observing phenomena on the basis of a certain amount of supporting evidence or the exploratory pursuit of observed findings (Guyatt et.,al 2011). Without such context, differing levels of observational evidence will presumably and often will be observed to be accepted to the same level of certainty, with the greater proven evidence to demonstrate functional value of the soils and management potential of those soils and their microbial inhabitants. The analysis of sequencing data can be very indicative of key relationships and processes in microbiomes: agricultural usefulness of an observed association often depends on its demonstration in a plethora of production systems and environments. Thus, merely having a biological association does not ensure that it can facilitate dependable agricultural decision-making. A biological association identified in a controlled experiment may provide important scientific insight, yet its agronomic significance may remain uncertain until it has been evaluated across multiple locations, seasons, and production systems. Although published studies frequently describe associations between metagenomic indicators and ecosystem

functions, comparatively few provide clear guidance on the level of evidence required before those indicators can support agricultural decisions (Louca et al., 2018; Prosser and Nicol, 2008). Consequently, a formal evidence hierarchy is required to distinguish between observed biological relationships, validated agronomic outcomes, and deployable recommendations (Guyatt et al., 2011).

3.2. Adaptation of the GRADE Framework for Soil Metagenomics:

The GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework was created within evidence-based healthcare to assess the quality of evidence and the strength of recommendations effectively (Guyatt et al., 2011). Throughout the years, evidence-driven methods have been utilized in numerous areas where suggestions should be determined by both the quality of the existing evidence and the trust that can be established in it. However, direct application of the original GRADE framework to environmental genomics is challenging because the sources of uncertainty differ substantially from those encountered in clinical research. VMDAR adjusts the GRADE framework principles to better align with the realities of soil metagenomics and precision agriculture. Instead of depending on clinical hierarchies, the framework emphasizes field validation, regional calibration, reproducibility, and monitoring of deployment. Every category of evidence is associated with specific deployment conditions, guaranteeing that recommendations are applied only at a level backed by the existing evidence. This is especially significant as biological plausibility by itself does not consistently lead to reliable agronomic performance in field conditions (Witt and Dawe, 2004; Vanlauwe et al., 2010). There may also need to be an E4 category that provides space for new indicators, formulas, and concepts of research for which there is limited published evidence of utility. Such use ensures that innovative ideas are not unduly adopted while their potential as a subject for future scientific investigation remains. Rather than traditional evidence scoring schemes where the strength of a recommendation depends heavily on the evidence used to develop it, VMDAR requires that evidence from at the evidence must meet prescribed minimum levels before a recommendation can proceed, placing evidence Level as its principal criterion for deployment. Accordingly, no combination of high-quality data, seasonal consistency, or spatial precision can compensate for inadequate evidence of support. This precautionary approach is intended to reduce the risk of deploying biologically plausible but insufficiently validated agronomic recommendations (Guyatt et al., 2011).

3.3. Classification of Evidence for Validation and Deployment:

The VMDAR framework classifies evidence into four categories (E1-E4) representing progressively lower levels of validation and deployment of readiness. The distinction between these categories is based not only on scientific confidence but also on the extent to which a finding has been independently validated under relevant agronomic conditions. Consequently, the evidence hierarchy serves both as a scientific classification system and as a governance mechanism that determines whether observation, interpretation, recommendation, or prescription can progress toward operational deployment (Guyatt et al., 2008; Guyatt et al., 2011).

3.4. Scope and Applicability of the VMDAR Framework:

The purpose of VMDAR is to provide a practical framework for evaluating when environmental genomic observations are sufficiently supported to inform agronomic recommendations. Although examples in this review are drawn from soil metagenomics, the framework is not limited to a particular indicator, crop, sequencing platform, or recommendation system. Instead, it focuses on the process of assessing evidence of quality, validation of status, confidence, and readiness for deployment in agricultural decision-making. Many types of biological information may benefit from this approach, including indicators derived from metagenomics, meta transcriptomics, microbial ecology, functional gene profiling, and soil health assessment. The same principles may also be applied to nutrient-management advisories, disease-risk evaluations, soil health monitoring programs, and other biologically informed decision-support systems. As new environmental genomics technologies emerge, the framework can be adapted to accommodate additional forms of biological evidence. For this reason, VMDAR should be viewed as a general validation and decision-support framework rather than a system developed for a single methodology or biological index. Its primary role is to promote consistent evidence of evaluation, transparency in recommendation development, and accountability in the deployment of genomics-based agronomic guidance.

4. Hypotheses And Pre-Defined Acceptance Standards:

There is a common tendency in emerging environmental genomics applications to establish success criteria only after results have been obtained. This type of decision-making after the fact raises the likelihood of confirmation bias and undermines the reproducibility of validation research. To reduce this risk, the VMDAR framework demands that all statistical acceptance standards, deployment limits, and promotion criteria be defined prior to the start of experimentation. Any changes to these criteria following the start of the study necessitate a formal evaluation, documentation, and approval via the governance process of the Scientific Advisory Board (SAB). This method connects recommendation implementation to established evidentiary criteria rather than looking back at the interpretation of outcomes (Guyatt et al., 2011).

4.1. Adequacy of Sequencing Depth:

There is an ongoing practical challenge in applied soil metagenomics related to balancing sequencing depth with operational costs. Deep sequencing might provide greater sensitivity; nonetheless, extra analytical information is only warranted when it results in significant advancements in biological insight or recommendation accuracy. Conversely, if lower sequencing depths provide comparable diagnostic results, increased sequencing could incur unnecessary expenses

without clear agronomic benefits. As a result, this section evaluates if intermediate sequencing depths offer adequate information for dependable biological index creation and pathogen identification. The objective isn't simply a quality assessment of reads counts but to quantify the minimum level of sequencing at which the interpretations or advice given remain consistent. Analogous questions relating to both the adequacy of sequencing and that of the analysis have already been posed for the fields of environmental genomics and microbial community ecology. It has generally been shown that in such fields, increasing the level of sequencing beyond a given threshold increases biologically informative data, to a diminishing degree, with each increase (Caporaso et al., 2012, Quince et al., 2017, Fierer, 2017). The defined acceptance criteria aim to determine if cost savings are possible while retaining a level of biological validity that provides diagnostic value.

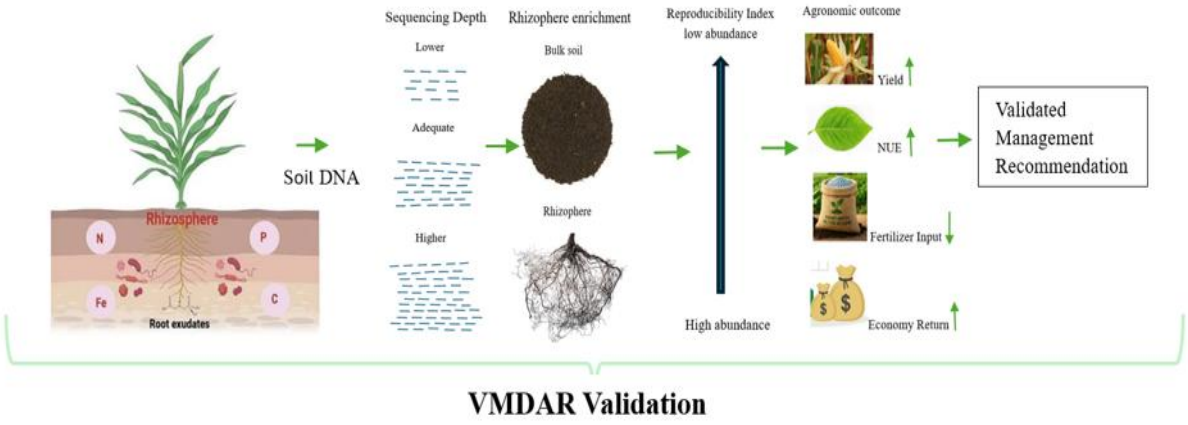

Figure 2. VMDAR Validation and Deployment Framework for Agronomic Recommendations:

Illustration of VMDAR validation showing the sequencing, ecological, analytical, and agronomic processes involved in vetting soil DNA information before producing actionable management advice.

4.2. Gradiant of Rhizosphere enrichment:

The Tri-Zone Ecological Model has a sound scientific basis rooted on the premises that biological functions evolve harmonically across the continuum soil-microorganisms (bulk soil, root-zone soil and rhizosphere soil) (Hinsinger et al., 2009; Philippot et al., 2013). Though Rhizosphere enrichment is widely shown in research there is variability regarding the frequency or amplitude of enrichment depending on crop species, soil type, environmental context (Philippot et al., 2013; Berendsen et al., 2012). Hence this section will assess whether the proposed tri-zone sampling design offers ecologically meaningful gradients that exhibit consistency across different soil types and crop types. The demonstration of progressive improvement trend would strengthen biological relevance of Delta Index and sampling design altogether. Conversely failure to demonstrate such gradient might suggest the need for improvement in the sampling model before operational use. The criteria places equal weight to the statistical confidence of the index and the replication of the patterns in relevant real conditions (Berendsen et al., 2012; Hinsinger et al., 2009).

4.3. Reproducibility of the index:

An essential attribute for a decision-support tool that would be used to make recommendations is consistency over analytical measurement repetition.

A significant variation in results after multiple replicate measurements of the same sample is not reliable enough to support agronomic recommendations. Given the multiple steps involved (DNA isolation and library preparation, sequencing and bioinformatics analysis), variability is to be expected (Bustin et al., 2009). In this section, it will be assessed whether the selected biological index (Delta Index) exhibits robustness to these technical variations in the different steps. Our intention is to determine whether the variation would indicate meaningful biological differences, or if it is rather methodological noise. The criteria are based on the same logic applied to the validation of measurements in clinical settings, environmental monitoring and quality control systems (ISO/IEC 17025:2017; Bustin et al., 2009).

4.4. Agronomic superiority of bio-integrated recommendations:

Beyond proving its ability to detect signals, the value of environmental genomics for agriculture relies on its ability to promote superior agronomic results in actual field conditions. A tool that identifies microbial indicators that might signify functional processes should eventually contribute to improve nutrient use efficiency, maintaining yield or enhance farmer income. The current question is indeed the most significant one from the current validation exercise because it addresses the potential value added of the biological signals detected when incorporated in integrated recommendations; i.e., the ability of bio-integrated recommendations to support as effective an agriculture as is currently practiced while reducing the demand on artificial inputs. Similar questions addressed relevant integrated nutrient management practices (Vanlauwe et al., 2010) and site-specific recommendations (Witt and Dawe, 2004; Arulmani et al., 2025) leading to farmers acceptance. Therefore, VMDAR assesses recommended suitability based on

yield consistency, nutrient use efficiency and economic returnability (Vanlauwe et al., 2010; Witt and Dawe, 2004; Arulmani et al., 2025). Figure 2 summarizes, the relation between the proposed validation criterion (sequencing, ecological validation, reproducibility of the index and agronomic evaluation).

5. Validation And Deployment Pathway For Agronomic Recommendations:

The VMDAR framework offers transparency to help bridge observed biological issues and agronomic recommendations via an evidentially driven validation process. The VMDAR thus comprises eight linked steps of the recommendation lifecycle and these span evidence creation, calibration, field trials and governance before going live.

Table 1. Eight-Step Recommendation Lifecycle in the VMDAR Framework:

Step	Validation Stage	Key Activity	Review Gate	Primary Output	References
1	Literature Validation	Systematic review of published evidence and initial E1-E4 assignment	SAB Microbiologist	Evidence classification	Guyatt et al., 2011; Guyatt et al., 2008
2	Soil-Series Baseline Mapping	Establish biological reference intervals and spatial calibration dataset	SAB Soil Scientist + Biostatistician	Calibrated reference intervals	Ettema & Wardle, 2002; Vos et al., 2013; Cressie, 1993
3	Threshold Calibration	Validation against physical reference measures and empirical weighting	SAB Biostatistician	Calibrated biological indices	Deel et al., 2024; Jolliffe, 2002; Witt and Dawe, 2004
4	Controlled Pilot Trials	Single-location, single-season field evaluation	SAB Agronomist	Preliminary agronomic performance report	Gomez & Gomez, 1984; Piepho et al., 2011
5	Multi-Location RCBD Trials	Full agronomic validation across locations and seasons	Full SAB Review	RCBD validation report	Arulmani et al., 2025; Murthy et al., 2024; Piepho et al., 2011
6	Independent Validation	Blind re-analysis using external datasets and institutions	Independent University Partner	Independent validation certificate	ISO/IEC 17025:2017; Bustin et al., 2009
7	Government Pilot Testing	District-levelI would also like to mention that exceptional permission was obtained for the cash payments made under these circumstances. implementation under controlled conditions	State Agriculture Representative	Government pilot evaluation report	Vanlauwe et al., 2015; Witt and Dawe, 2004
8	State-Scale Deployment	Formal approval and post-deployment monitoring	SAB Supermajority Vote	Operational advisory system	Guyatt et al., 2011; Witt and Dawe, 2004

6. Statistical Acceptance Criteria — Full Specification:

The statistical validation criteria established within the VMDAR system were used to assess the performance and reliability of biological indices and advisories prior to implementation, as to justify their use based on evidence from measurement before deployment.

Table 2. Pre-Defined Statistical Acceptance Criteria for the Validation of Biological Indices:

Index	Validation Measure	Acceptance Criteria	References
	Crop N uptake	$R^2 \geq 0.70$	Herridge et al., 2008

NAP	(^15N isotope dilution) N mineralization rate Prediction accuracy PGPR filtering (waterlogged paddy)	 $R^2 \geq 0.65$ RMSE ≤ 15 kg N ha^{-1} ≥95% reduction in Clostridium and Desulfovibrio attribution	Prosser and Nicol, 2008 Deel et al., 2024; Piepho et al., 2011 Gaby and Buckley, 2014; Tu et al., 2019
PMP	Olsen P (pH ≥ 6.5) Bray-1 P (pH < 5.5) Prediction accuracy pH modifier validation	$R^2 \geq 0.70$ $R^2 \geq 0.70$ RMSE ≤ 5 kg P_2O_5 ha^{-1} Negative phoD coefficient in acidic soils	Olsen et al., 1954; Sparks, 2003 Bray and Kurtz, 1945; Sparks, 2003 Deel et al., 2024; Piepho et al., 2011 Ragot et al., 2015; Mander et al., 2012
DPI	Diagnostic sensitivity Diagnostic specificity False positive rate Calibration dataset CRITICAL + HIGH sensitivity	≥85% ≥ 90% <5% ≥200 DPI-DSI pairs; ≥3 soil series; ≥5 pathogens ≥90%	Bustin et al., 2009; Piepho et al., 2011 Bustin et al., 2009; Piepho et al., 2011 Bustin et al., 2009 Gomez and Gomez, 1984; Piepho et al., 2011 Piepho et al., 2011

Table 3. Crop-Specific Evidence Levels, Validation Requirements, and Agronomic Acceptance Criteria for Recommendation Deployment:

Crop	Current Evidence Level	Minimum Validation Requirement	Agronomic Threshold	References
Soybean	E1	Annual verification on ≥20 farms	Yield ≥ 95% of RDF	Herridge et al., 2008; Arulmani et al., 2025; Murthy et al., 2024
Paddy/ Rice	E2	≥3 districts; ≥3 seasons; ≥100 plots	Yield ≥ 95% RDF; NUE +15%	Biswas et al., 2000; Witt and Dawe, 2004; Vanlauwe et al., 2010
Chickpea	E2	≥2 locations; ≥2 seasons; ≥80 plots	Yield ≥ 95% RDF; P availability ≥90% control	Zaidi et al., 2009; Vanlauwe et al., 2010
Maize	E2	≥2 locations; ≥4 seasons; ≥100 plots	Yield ≥ 95% RDF; no K depletion	Meena et al., 2014; Witt and Dawe, 2004
Wheat	E3	≥3 agro-climatic zones; ≥3 seasons	No fertilizer reduction before E2 promotion	Prosser and Nicol, 2008; Louca et al., 2018
Potato	E3	≥2 seasons; ≥2 locations (Indian evidence)	Pathogen diagnostics only	Mendes et al., 2011; Weller et al., 2002

6.1 Factors governing adoption of government:
VMDAR has a number of predefined acceptance thresholds which are important in rigorously assessing whether the proposed framework is viable for application in the agricultural environment. Value-Cost Ratio (VCR) has to be above 2.0 and Cost-Benefit ratio (CBR) should be above 4.0 to show positive net benefit against the costs associated in implementation of proposed framework. Limit of detrimental yield results should be 5% across pilot farms and farmer should have at least 80% of satisfaction rate for acceptability and yield performance should be at 90% in contrast to RDF controlled one.

7. Quality Control Standard For Validation Experiments:
The success of metagenomic analysis highly depends on the quality of the sequencing obtained and methods adopted in the workflow. Several research group stress the importance of the application of controls on sample and in several step of data process for finding the contaminants in the experimental material. Evaluating methodological rigor and increase overall confidence level about any subsequent finding has been indicated as the role of controls. (Salter et al., 2014; Eisenhofer et al., 2019). Consequently, validation experiments conducted within the VMDAR framework include negative controls, positive controls, spike-in standards, and technical replicates as elements of regular quality evaluation (Hardwick et al., 2018; Tourlousse et al., 2017). Quality-control assessment encompasses more than just contamination checks; it also includes sequencing performance, analytical consistency, and variation from batch to batch. Evaluating these elements prior to biological interpretation aids in differentiating authentic biological trends from technical artifacts and enhances the resilience of index creation (Callahan et al., 2016; Bokulich et al., 2013). If quality metrics exceed established acceptance limits, the cause of the variation is examined, and extra verification measures might be implemented before the outcomes are considered for deeper analysis. Within the VMDAR framework, these processes aid in producing dependable datasets that facilitate the creation of biological indices and the following agronomic interpretation. Uniform implementation of quality-control procedures also enhances reproducibility among experiments, laboratories, and sequencing batches, which is still a crucial necessity for practical metagenomic applications (Bustin et al., 2009; ISO/IEC 17025:2017).

Table 4. Laboratory Quality-Control Standards for Validation Experiments:
The acceptance thresholds shown in this table represent proposed VMDAR quality-control criteria informed by published microbiome quality-assurance practices and laboratory validation standards.

QC Component	Procedure	Acceptance Criteria	Action if Failed	References
Negative Controls	Extraction blanks processed with every batch Library preparation blanks processed with each library batch	<100 reads (16S); <50 reads (ITS) Below predefined contamination threshold	Batch investigation and possible rejection Batch investigation and possible rejection	Salter et al., 2014; Eisenhofer et al., 2019 Salter et al., 2014; Eisenhofer et al., 2019
Positive Controls	Mock microbial community (e.g., ZymoBIOMICS standard) included in every extraction batch	≥95% species recovery; relative abundance within ±15% of expected composition	Repeat processing and QC review	Yeh et al., 2018; Tourlousse et al., 2017
Spike-in Controls	Synthetic DNA standards (e.g., meta-sequins) added at graded concentrations	Establish batch-specific limit of detection (LOD) and limit of quantification (LOQ)	Recalibration and repeat validation if required	Hardwick et al., 2018; Tourlousse et al., 2017

Reproducibility Testing	Triplicate DNA extractions performed on 10% of validation samples	Coefficient of variation (CV) reported for each biological index	Investigating analytical variability and repeat extraction if necessary	Bustin et al., 2009; ISO/IEC 17025:2017
Sequencing Quality Control	Sequencing run performance monitored using platform controls	≥80% bases with Q30 or higher; run metrics within manufacturer specifications	Repeat sequencing run or exclude affected samples	Quince et al., 2017; Goodwin et al., 2016
Bioinformatics Quality Control	Quality filtering, chimera removal, and taxonomic assignment validation	≥90% reads retained after filtering; negligible chimera rate	Review analysis pipeline and repeat processing	Callahan et al., 2016; Bokulich et al., 2013
Cross-Batch Validation	Inclusion of common reference samples across batches	No significant batch effect detected ($p > 0.05$)	Batch correction or reanalysis required	Leek et al., 2010; Nygaard et al., 2016
Data-Integrity Verification	Sample metadata and chain-of-custody review	100% metadata completeness and traceability	Metadata correction and audit review	ISO/IEC 17025:2017

7.1. External Validation and Cross-Platform Verification:

The framework incorporates external validation to determine whether its output remains consistent when evaluated using different sequencing platforms and analytical environments. Accordingly, a minimum of 10 soil samples from each Phase 1 validation batch shall undergo independent processing at a second laboratory using an alternative NGS platform (e.g., Illumina short-read sequencing and Oxford Nanopore long-read sequencing). Validation acceptance requires at least 90% agreement in HIGH, MEDIUM, and LOW classifications for both NAP and PMP. Results that fail to meet this criterion shall undergo further platform-level investigation prior to Phase 1 advisory deployment.

8. Comparison of VMDAR with Existing Evidence and Recommendation Frameworks:

Existing frameworks address specific aspects of evidence evaluation, soil health assessment, fertilizer recommendation, or analytical validation. However, they generally do not integrate evidence classification, reproducibility assessment, confidence evaluation, governance oversight, and deployment readiness within a single framework. VMDAR was developed to address this gap by providing a structured pathway that links metagenomic observations to agronomic recommendations through predefined validation and decision-making processes. At present, VMDAR should be regarded as a conceptual and methodological framework, and future studies should evaluate its performance through real-world application and deployment case studies.

Table 5 Comparison of VMDAR with Existing Evidence and Recommendation Frameworks:

Framework	**Primary Purpose**	**Evidence Assessment**	**Analytical Validation**	**Confidence Assessment**	**Governance Oversight**	**Demonstrated Deployment**
GRADE	Clinical evidence evaluation	Yes	No	Yes	Limited	No
STCR	Soil test-based fertilizer recommendations	Partial	Yes	No	No	Yes
Soil Health Assessment Frameworks	Soil health evaluation	Partial	Partial	Limited	No	No

Diagnostic Validation Frameworks	Assay and method validation	Partial	Yes	Limited	No	No
VMDAR	Metagenomics-driven agronomic recommendations	Yes	Yes	Yes	Yes	Proposed future application

9. Specification Of Bioinformatics Pipeline:

This review proposes a standardized bioinformatics workflow to facilitate taxonomic filtering, functional gene annotation, chemistry-substitution verification, and change-management practices within the VMDAR framework. In the accompanying tables, required operating parameters, evaluation criterion and implementation factors are summarized. The information herein summarizes the specification of key elements that guide methodological procedures in developing metagenomic indicators. The overall functional gene annotation workflow employed within the VMDAR framework is illustrated in Figure 3.

9.1. nifH Taxonomic Filtering Workflow for NAP Computation:

The VMDAR framework employs a standardized taxonomic filtering workflow to improve the biological relevance of nifH-based Nitrogen Availability Potential (NAP) estimation. Initially, all metagenomic reads are aligned against the NCyc v2.0 and KEGG Orthology databases using DIAMOND with predefined alignment quality thresholds, including an e-value of $<1\times10^{-5}$, bitscore ≥50, and query coverage $\geq70\%$ (Kang et al., 2019). Taxonomic assignments are subsequently generated for nifH-positive reads using the best-hit approach at the genus level (Kang et al., 2019). To filter the analysis towards agriculturally important biological nitrogen fixation, the VMDAR keeps only the documented beneficial or plant-growth-promoting or diazotrophic bacteria. In the following, the genera symbiotic N fixers selected are Bradyrhizobium, Sinorhizobium, Mesorhizobium, Rhizobium, and Azorhizobium (Herridge et al., 2008). The genera free-living or associative diazotrophs kept within the frame are Azospirillum, Herbaspirillum, Gluconacetobacter, and Azoarcus (Gaby and Buckley, 2014). For flooded rice production systems, cyanobacterial nitrogen-fixing genera such as Anabaena, Nostoc, and Cylindrospermum are additionally retained due to their established contribution to biological nitrogen fixation in paddy ecosystems (Roger and Ladha, 1992). Conversely, non-target and agriculturally less relevant nifH-carrying genera are excluded from NAP computation. These include anaerobic taxa such as Clostridium, Desulfovibrio, Desulfosporosinus, and Sulfurospirillum, as well as all genera not included in the validated diazotroph list (Gaby and Buckley, 2014; Tu et al., 2019). To ensure adequate biological support for index generation, VMDAR further requires a minimum threshold of 1,000 PGPR-filtered nifH reads before NAP computation proceeds. Samples failing to satisfy this requirement are considered to have insufficient biological signal for reliable NAP estimation and are flagged accordingly as part of the framework's operational specifications.

9.2. Functional Gene Annotation Pipeline Specifications:

The VMDAR framework utilizes a standard pipeline for functional gene annotation in its metagenomic analyses. Sequence alignment to KEGG Orthology (KO), NCyc v2.0, and Fungal Traits databases are performed using DIAMOND v2.0, which assist the annotation of microbial metabolic capabilities, nitrogen-cycling genes, and fungal ecological attributes (Kang et al., 2019; Kanehisa et al., 2023; Plme et al., 2020; Buchfink et al., 2021). Based upon the results from quality-filtered sequences alignment the functional gene assignments to KO level are done by a best hit approach. Within the VMDAR the output from DIAMOND are evaluated in terms of e-value, bit-score, query coverage and other threshold to achieve consistency in analyses. In VMDAR pipeline the genes abundance should be reported as relative abundance to enable comparison between samples, and the total abundance is scaled by using Total Sum Scaling (TSS) approach (Weiss et al., 2017). Genes below the defined detection threshold were excluded from the analysis to reduce false discoveries. The output is a KO-level functional abundance table which could be used to develop biological indices, validate their effectiveness, and implement on-farm recommendations (Kanehisa et al., 2023; Kang et al., 2019).

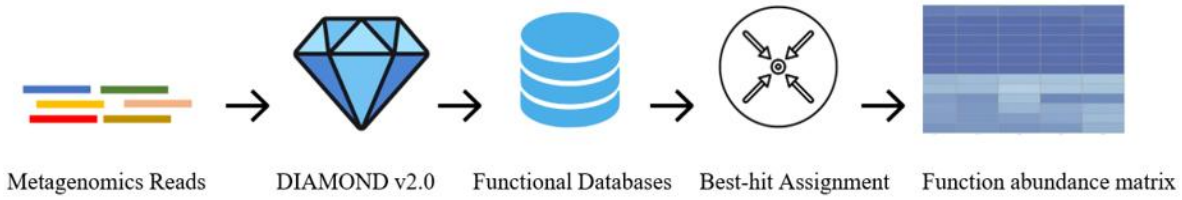

Figure 3. Functional gene annotation flow:
This section outlines the pipeline sequence used in the VMDAR framework to annotate genes. Data is aligned using DIAMOND against KO, NCyc, and Fungal Traits databases; followed by functionally assigning, normalization and quality filtering of each data.

9.3. Version-Control and Change-Management Requirements:
All database versions and associated processing thresholds used in the VMDAR framework shall be formally recorded in the Laboratory Information Management System (LIMS) at the time of analysis. Any change to databases, sequence alignments, filtering, or processing steps require a formal change request and scientific review prior to implementation. The framework shall document how any changes impact or potentially impact the validity of existing biological indices and Recommendations. The framework shall also include requirements for comparison of analytics prior and post changes using a specific set of validation samples or dataset to compare the existing indices and Recommendations against the new proposed procedure. Final approval for all change requests shall be completed through the governance process prior to making these changes available for operational use.These procedures shall be consistent for laboratory information management systems (LIMS) in terms of the transparency, traceability, reproducibility and quality assurance process required for validation studies (ISO/IEC 17025:2017).

9.4. Chemistry Substitution Validation Workflow:
The framework shall demonstrate how conventional chemical analytical measures can be replaced by metagenomic analogues through the validation of the following: (Witt and Dawe, 2004; Breiman, 2001, Piepho et al., 2011, James et al., 2021; Deel et al., 2024). Parallel collection of both traditional chemistry based and metagenomic based soil samples for comparison. Establishment of regression model/s (i.e., linear regression and random forest) on parallel collected data and its' prediction capacity shall be assessed using the Coefficient of Determination (R), the Root Mean Square Error (RMSE), and the Mean Absolute Error (MAE) between the independent variable and the predicted variable.
Development of and testing statistical validation methodologies, for example, using hold-out validation. For example, 80% of the data will be used for training and validation, and hold-out validation, with prediction performance evaluated using CVR and RMSE. Evaluation of and defining Acceptance Criteria based upon the predictive ability demonstrated by the models. The substitution of chemistry-based measures with metagenomic-derived proxies should not be pursued until sufficient data has accumulated from validation studies to provide confidence in the quality, consistency, and replicability of the metagenomic indicator. (Vanlauwe et al., 2010).

9.5. Eligibility of Chemistry Parameters for Metagenomic Substitution:
Soil pH, EC and K are kept as primary parameters which are retained permanently (Sparks, 2003). N, P, SOC and MBC can potentially be substituted using a metagenomic approach, these parameters need validation prior to their inclusion (Deel et al., 2024; Witt and Dawe, 2004; Vanlauwe et al., 2010; Lehmann et al., 2020; Vance et al., 1987).

10. SAB Governance And Operational Procedures:
The SAB (Scientific Advisory Board) offers scientific independent scrutiny and advice to the validation and deployment elements in the VMDAR framework and ensures that recommendations address established criteria regarding evidence, quality assurance, analytical validity, risk assessment and deployability.

10.1. Constitution and Operational Structure of the SAB:
The SAB will be constituted before formal validation activities and will comprise multidisciplinary expertise in soil science, agronomy, microbiology, biostatistics, policy implementation, and farmer representation. Transparency The panel will provide expert critique and analysis that support clear, responsible and scientific decision making about study designs, evidence requirements, validation, and their placement in current clinical practice (Guyatt et al., 2011).

10.2. Decision-Making and Voting Requirements:
Major decisions, including protocol approval, validation sign-off, recommendation deployment, suspension, and regulatory certification, will follow predefined voting requirements. The depth of the review will reflect the importance of each decision regarding their Scientific impacts, being the higher risks and therefore the greater level of review (Guyatt et al., 2008; Guyatt et al., 2011).

10.3. Deployment Petition Review Process:
To operational deployment, you will submit a Deployment Petition to the SAB. This petition will include findings of validation studies, literature reviews, field trial results, risks, suggested wording for advisory products, and operational post deployment monitoring procedures. Deployment will be authorized only when the submitted evidence satisfies predefined acceptance criteria.

10.4. Monitoring of Negative Incidents and Remedial Measures:
Post-deployment monitoring will be used to identify unexpected or adverse outcomes under field conditions. Incidents identified shall be examined as to their cause and their effects both on and off the farm, recommendations shall be altered, suspended temporarily or withdrawn if needed. This is an important quality for maintaining the utility of the framework over time, supporting it's dependable, continued usage, and facilitating adaption in response to evolving circumstances (Vanlauwe et al., 2010; Guyatt et al., 2011).

10.5 Reproducibility and Inter-Laboratory Validation:
Reproducibility should be assessed to distinguish between biological variability and variability introduced during DNA extraction, library preparation, sequencing, and bioinformatic processing. The VMDAR framework should guide sequencing consistency checks, with the intention to test between-batch reproducibility, within-batch reproducibility, and agreement across independent labs. Failure of reproducibility will result in a penalty against the Overall Confidence component within the Data Quality value which directly translates to the analytical uncertainty incorporated into recommendation confidence (Bustin et al., 2009; ISO/IEC 17025:2017; Goodwin et al., 2016).

10.6 Criteria for Machine Learning Verification:
Machine learning validation within VMDAR should progress from simple, interpretable statistical models to more complex algorithms based on performance needs and intended application. If models are intended for operational use, they should undergo rigorous evaluation against prespecified performance and validation criteria, and reporting should include specification of the training and validation dataset, performance measures, calibration, key features, and limitations. This stepwise approach enhances interpretability and reduces potential overfitting, making model functionality accessible to the SAB for deployment consideration (Breiman, 2001; James et al., 2021).

11. Limitations And Future Work:
Like any framework, VMDAR has shortcomings that must be addressed through continuous testing and improvement. Currently, the framework provides a methodical process for determining when metagenomic insights may support agronomic recommendations, but a number of considerations warrant validation in various field conditions. Every agricultural system varies factors such as soil properties, climate, cropping patterns and management history will impact the reliability of biological indicators and associated agronomic recommendations.
Another important challenge arises from the inherent dynamic nature of soil microbial communities. Biological signals can shift significantly by season, geography, and management practice, meaning patterns observed in one context are not necessarily transferable to another. The need for continuous recalibration and independent validation will therefore persist as new datasets accumulate. Successful deployment of metagenomics-based decision support is contingent on representative sampling, robust sequencing data, and standardized bioinformatics pipelines; analytical variations (e.g. Differences in lab processes, sequencing technology or bioinformatic workflow) impacting reliability of biological metrics should be periodically assessed. Future work should prioritize extensive field validation in various agro-ecological zones, long-term index consistency monitoring, and a wider network of inter-laboratory comparative studies. Additionally, enhancements will be required to increase confidence scores, improve model interpretability, and extend the utility of the framework to other novel environmental genomics technologies. As additional validation data become available, the VMDAR framework can be further adapted and strengthened to support a broader spectrum of precision agriculture and soil health decision-making in diverse contexts.

12. CONCLUSION:
Soil metagenomics have gained a tremendous amount of interest as they represent an approach for unearthing the biological activity within agricultural soil ecosystems. Numerous studies have reported correlation between soil microbial community structure and functionally important properties, paving the way for utilization in an agricultural context. However, extrapolating from biological discoveries to applicable recommendations has proven far from straight forward. Correlations established in one scenario may simply not hold in another and require stringent validation to become actionable advice. We propose the VMDAR framework as a mechanism to address the gap between the identification of useful soil biological indicators and their deployment in agricultural recommendations by structuring the evaluation of evidence, the evidence base in terms of its validation status and associated confidence. Combining these parameters in a single framework would provide a clear path from soil microbial observations to agronomic actions. VMDAR incorporates a systematic evidence framework, defined acceptance criteria, reproducibility mandates, and oversight from a Scientific Advisory Board to ensure that guidelines have adequate scientific support before being put into practice. Although developed for soil metagenomics, the underlying principles of VMDAR may be applicable to a broader range of environmental genomics and biological monitoring systems. As metagenomic technologies continue to advance, frameworks that emphasize transparency, validation, and deployment of readiness will be essential for converting biological information into reliable, evidence-based agronomic recommendations.

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BD contributed to the study of conception and design. Material Preparation was performed by PD, CA, VB, HM, MR, RD. BD read and approved the final manuscript.

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The authors declare no conflict of interest.

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