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# DEVELOPING A RHIZOSPHERE BIOMARKER PANEL TO BREAK THE NITROGEN-SHEATH BLIGHT LOOP IN INTENSIVELY IRRIGATED PADDY

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## ABSTRACT
The rhizosphere is the narrow but biologically active zone of soil influenced by living roots. It is not simply a reservoir of nutrients; rather, it functions as a dynamic biochemical interface where roots release sugars, amino acids, organic acids, phenolics, enzymes and other compounds that regulate microbial communities and nutrient transformations. The development of a reliable biomarker panel requires substantial validation across rice-growing environments. The experiment was designed to determine whether a rhizosphere biomarker panel can identify and predict the development of sheath blight under different nitrogen and irrigation regimes and, subsequently, be used to optimize nitrogen management and reduce disease severity without compromising rice yield. The experimental framework demonstrated that the nitrogen–sheath blight relationship in intensively irrigated paddy should be interpreted as a combined soil, rhizosphere, plant and pathogen phenomenon rather than as an isolated disease problem. The biomarker panel provided a multidimensional approach for assessing nitrogen availability, biological nitrogen cycling, pathogen pressure, beneficial microbial activity and the disease-suppressive capacity of the rhizosphere. This approach is particularly relevant to irrigated rice because nitrogen-use efficiency is generally low, with the biomarker document indicating that only about 30–40% of applied nitrogen is typically recovered by the crop, while the remainder may be lost through nitrification–denitrification and volatilization. A rhizosphere biomarker panel offers such an approach by providing measurable biological and biochemical indicators of nitrogen availability, microbial activity, plant nutritional status, pathogen pressure and disease-suppressive capacity before visible symptoms become severe. Biomarker monitoring can therefore help distinguish beneficial biological activation from uncontrolled nutrient release. An important advantage of the biomarker approach is its potential for precision nutrient and disease management.

**KEYWORDS:** Rhizosphere, Biomarker Panel, Nitrogen–Sheath Blight Loop, Irrigated Paddy.

## 1.INTRODUCTION
Intensively irrigated paddy systems are among the most productive agricultural systems in the world, but their high productivity is frequently accompanied by excessive nitrogen fertilization, prolonged soil wetness, dense crop canopies, and favourable microclimatic conditions for disease development (Ganesh and Roka, 2024; Kumar et al., 2014). In flooded rice soils, the rhizosphere is further modified by oxygen release from roots, redox gradients, iron and manganese transformations, nitrogen mineralization and denitrification, and the continuous exchange of carbon and nutrients between plants and microorganisms (Kaviraj et al., 2024; 2025; Sahoo et al., 2022). These processes determine whether nitrogen is efficiently captured by rice or lost through leaching, volatilization, and gaseous pathways, and they also influence the microbial environment in which soil-borne pathogens compete with beneficial microorganisms (Ghosh et al., 2025; Guo et al., 2024). Nitrogen is an essential element in this system. Adequate nitrogen is essential for leaf-area development, chlorophyll formation, protein synthesis, tillering and yield formation. However, excessive or poorly synchronized nitrogen application can produce an excessively dense canopy with succulent tissues, increased humidity within the crop, and prolonged periods of leaf and sheath wetness (Brajendra et al., 2012; Khan et al., 2025; Varanavasiappan et al., 2024). These conditions can favour sheath blight development. High nitrogen availability may also alter plant carbon allocation and tissue chemistry, potentially influencing the plant's capacity to tolerate or restrict pathogen invasion. Consequently, the objective should not simply be to reduce nitrogen fertilizer, but to synchronize nitrogen supply

with crop demand while maintaining a rhizosphere environment capable of supporting nutrient-use efficiency and disease suppression. The concept of a rhizosphere biomarker panel is based on the recognition that no single soil or plant measurement can adequately describe this complex interaction. Instead, a combination of biomarkers can provide a biological fingerprint of the field. Such a panel may include soil mineral nitrogen, potentially mineralizable nitrogen, microbial biomass carbon and nitrogen, soil respiration, extracellular enzyme activities, pH, redox status, dissolved organic carbon, available phosphorus and potassium, plant tissue nitrogen, chlorophyll or canopy nitrogen indicators, root activity, pathogen abundance and indicators of beneficial microbial populations. The panel can be strengthened further through molecular measurements such as quantitative detection of R. solani, microbial community profiling, functional genes associated with nitrogen cycling, and biomarkers of microbial antagonism or disease suppression. The presence of microbial groups associated with nutrient cycling and biological suppression can also serve as indicators of soil health (Wang et al., 2024). Beneficial microorganisms, including species of Trichoderma, Bacillus, Pseudomonas, and other antagonistic or plant-growth-promoting organisms, may contribute to disease suppression through competition, antibiosis, mycoparasitism, production of hydrolytic enzymes, induction of plant defence and modification of the rhizosphere environment (Singh and Singh, 2025). Nitrogen biomarkers prevent unnecessary fertilizer accumulation, canopy and plant biomarkers identify excessive vegetative growth, pathogen biomarkers identify disease risk, and microbial biomarkers indicate whether the rhizosphere possesses biological capacity for nutrient cycling and disease suppression (Caon and Vargas, 2017; Rathod et al., 2021). A biomarker-based management system seeks to interrupt the loop at several points simultaneously. The biomarker panel can help determine how the rhizosphere responds to changes in irrigation and whether improved water management is accompanied by better nitrogen retention and reduced disease risk. Over time, repeated biomarker measurements could establish field-specific thresholds and improve the predictive accuracy of the system.

Rice varieties differ in canopy architecture, nitrogen responsiveness and sheath blight susceptibility. Soil texture, organic matter, flooding regime, temperature, irrigation practices and residue management also influence rhizosphere processes (Tuti et al., 2022; Parmar et al., 2022). Therefore, a biomarker threshold developed in one agroecological region should not automatically be applied to another. Multi-location experiments should compare nitrogen rates, irrigation regimes, resistant and susceptible cultivars, biological inputs and disease-management strategies while simultaneously monitoring rhizosphere biomarkers. Statistical modelling and machine-learning approaches can then be used to identify the smallest combination of indicators capable of predicting disease risk and nitrogen-use efficiency. Breaking the nitrogen-sheath blight loop is therefore fundamentally a problem of synchronization. Such an approach can reduce unnecessary fertilizer use, improve nitrogen-use efficiency, strengthen biological soil health and support more rational disease management. Therefore, the ultimate aim and the hypothesis behind this study were the development of a Rhizosphere Health and Disease Risk Index for irrigated rice. Such an index could combine nitrogen surplus, crop nitrogen status, microbial activity, pathogen abundance, soil moisture and biological suppression indicators into a single decision-support score. A farmer or extension worker could then interpret the field not simply as "nitrogen deficient" or "sheath blight infected," but as a biological system moving towards or away from a disease-favourable state. This represents a major conceptual shift from treating symptoms to managing system-level processes.

## 2. METHODOLOGY

### 2.1 Experimental objective

The experiment was designed to determine whether a rhizosphere biomarker panel can identify and predict the development of sheath blight under different nitrogen and irrigation regimes and, subsequently, be used to optimize nitrogen management and reduce disease severity without compromising rice yield. The main hypothesis was that excessive and poorly synchronized nitrogen supply under intensive irrigation modifies rhizosphere biological activity, plant nitrogen status and canopy microclimate in ways that increase Rhizoctonia solani development, whereas synchronized nitrogen and water management, supported by biomarker-guided decisions, can weaken this nitrogen sheath blight relationship.

### 2.2 Experimental site and soil characterization

A field experiments were conducted in an intensively irrigated rice-growing area with a documented history of sheath blight. Before land preparation, the experimental field was characterized for soil texture, pH, electrical conductivity, organic carbon, available nitrogen, phosphorus and potassium, mineral nitrogen fractions, bulk density and soil moisture characteristics. Initial soil samples were collected from 0-15 and 15-30 cm depths from representative locations using a stratified or zig-zag sampling procedure. Meteorological observations, including maximum and minimum temperature, relative humidity, and rainfall, were recorded throughout the experiment. Where possible, canopy temperature, relative humidity and duration of leaf/sheath wetness were also monitored because these variables are important determinants of sheath blight development.

### 2.3 Experimental Design

A factorial field experiment was established using a randomized complete block design or split-plot design, depending on the irrigation infrastructure (Gorlapalli et al., 2022). Four nitrogen-management treatments and three irrigation regimes were used to generate a gradient of nitrogen availability and rhizosphere conditions.

A representative nitrogen treatment structure could be:

**$N_1$ – Low nitrogen (**50% of the recommended nitrogen dose)
**$N_2$ – Recommended nitrogen (**100% of the recommended dose)
**$N_3$ – High nitrogen (**150% of the recommended dose)
**$N_4$ – Biomarker-guided nitrogen (**nitrogen applied according to crop demand and rhizosphere biomarker information)
The irrigation treatments could comprise:
**$I_1$ – Continuous intensive irrigation/flooding**
**$I_2$ – Intermittent irrigation with controlled soil water conditions**
**$I_3$ – Alternate wetting and drying or another locally validated water-saving irrigation regime**
The exact irrigation thresholds were determined according to soil type and local rice production recommendations rather than applying a universal water regime. Each treatment had at least four replications.

**2.4 Rice variety and crop establishment**

A commercially important rice variety with moderate susceptibility to sheath blight was selected for the experiment because a measurable disease gradient is necessary for evaluating the biomarker system. A resistant or relatively tolerant variety included as an additional treatment in a separate experiment to determine whether biomarker relationships remain valid across contrasting host resistance levels. Rice was established using the locally recommended transplanting or direct-seeding method. Plant spacing, seed rate, basal phosphorus and potassium fertilization, weed management, and other agronomic practices were maintained uniformly across treatments.

**2.5 Sheath blight inoculation and disease pressure**

Where natural disease pressure is reliably high and uniform, the experiment can depend on natural infection. However, for controlled experimental studies, artificial inoculation may be used to create a more consistent sheath blight pressure. A well-characterized R. solani isolate obtained from infected rice tissue will be cultured under appropriate laboratory conditions. Inoculum will be prepared using a validated experimental protocol and introduced at a standardized crop-growth stage. The inoculum dose and application method should be identical across inoculated plots. A separate non-inoculated control can be maintained where feasible to distinguish treatment effects on general rhizosphere and plant physiology from responses specifically associated with pathogen pressure.

**2.6 Development of the rhizosphere biomarker panel**

The biomarker panel should contain complementary indicators representing five major components: nitrogen availability, plant nitrogen status, rhizosphere biological activity, pathogen pressure, and disease-favourable environmental conditions.

2.6.1 Soil and Rhizosphere Nitrogen Biomarkers

The following parameters should be measured-

I. Ammonium-N
II. Nitrate-N
III. Potentially mineralizable nitrogen
IV. Microbial biomass nitrogen
V. Dissolved organic nitrogen
VI. Soil total nitrogen
VII. Nitrogen-use efficiency
VIII. Plant nitrogen uptake

Mineral nitrogen should be measured at important crop stages rather than only at harvest. This will enable the identification of periods when nitrogen availability exceeds crop demand.

2.6.2 Rhizosphere Biological Biomarkers

Rhizosphere soil should be collected carefully from soil adhering to actively growing roots. The following indicators may be measured:

I. Microbial biomass carbon
II. Microbial biomass nitrogen
III. Soil respiration
IV. Dehydrogenase activity
V. Urease activity
VI. Protease activity
VII. β-glucosidase activity
VIII. Phosphatase activity
IX. Potential nitrification activity
X. Functional microbial diversity

These measurements will provide an estimate of the biological activity and nutrient-cycling capacity of the rhizosphere (Figure 1).

2.6.3 Pathogen Biomarkers

The abundance of R. solani should be quantified using a validated molecular assay, preferably quantitative PCR targeting a species-specific or validated diagnostic marker. Rhizosphere soil, root-associated material and symptomatic sheath tissues can be sampled at defined crop stages.

The objective is to determine whether increases in pathogen abundance occur before visible disease symptoms and whether pathogen abundance interacts with nitrogen availability and canopy conditions.

2.6.4 Beneficial Microbial Biomarkers

Selected beneficial and potentially disease-suppressive microorganisms may be quantified using culture-dependent and/or molecular methods. Candidate groups can include Trichoderma, Bacillus, Pseudomonas and other taxa identified through preliminary microbiome analysis. For advanced experiments, 16S rRNA gene sequencing for bacterial communities and ITS sequencing for fungal communities can be used to characterize shifts in rhizosphere microbial diversity and community composition. The analysis should focus not only on microbial abundance but also on microbial functions associated with nitrogen cycling and disease suppression.

2.6.5 Plant Biomarkers

Plant measurements should include:

I. Leaf nitrogen concentration
II. Chlorophyll concentration or SPAD value
III. Leaf area index
IV. Number of tillers
V. Plant biomass
VI. Root biomass
VII. Root length and root activity
VIII. Canopy nitrogen status
IX. Photosynthetic performance, where facilities are available
X. Grain yield and yield components

These measurements will allow rhizosphere biomarkers to be related to actual plant nutritional status.

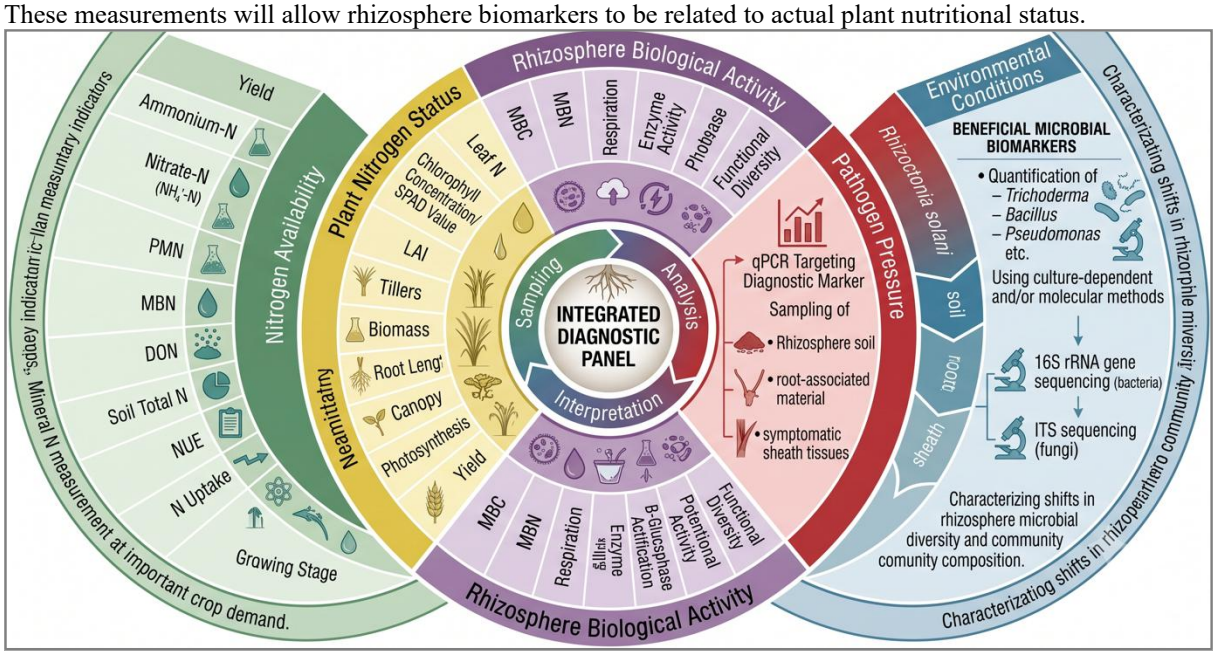


**Figure 1.** Conceptual diagram of development of a rhizosphere biomarker panel to break the nitrogen-sheath blight loop in intensively irrigated paddy

**2.7 Sampling schedule**

Sampling should be synchronized with major rice growth stages. A practical schedule would include:

**$T_0$ – Before transplanting or sowing (**baseline soil and rhizosphere characterization)
**$T_1$ – Early vegetative stage (**approximately 20–25 days after establishment)
**$T_2$ – Active tillering (**approximately 35–45 days)
**$T_3$ – Panicle initiation (**approximately 50–65 days)
**$T_4$ – Booting/heading (**approximately 70–85 days)
**$T_5$ – Grain filling (**approximately 90–105 days)
**$T_6$ – Harvest (**final soil, plant and yield assessment)

Sampling dates should ultimately be adjusted to the selected variety and local crop duration.

At each sampling event, soil/rhizosphere samples, plant tissue and disease observations should be collected from the same experimental plots. Repeated sampling of the same plots will permit temporal relationships among nitrogen availability, microbial activity, pathogen abundance and disease development to be established.

**2.8 Rhizosphere soil sampling procedure**

Three to five representative plants should be selected from the central portion of each plot to avoid border effects. Plants should be carefully excavated to minimize disturbance of the root system. Loosely adhering bulk soil should be removed, while soil closely associated with the roots should be collected as rhizosphere soil. Samples should be divided immediately according to analytical requirements. Fresh samples should be maintained under

appropriate refrigerated conditions for molecular and microbiological analyses, while separate portions should be processed for chemical and enzymatic assays. Samples intended for DNA analysis should be handled using contamination-minimizing procedures and stored under conditions appropriate for preservation of nucleic acids.

**2.9 Measurement and calculation of sheath blight**

Disease assessment should begin before visible symptoms become widespread and continue at regular intervals after inoculation or first disease detection.

Disease incidence will be calculated as equation 1-

**Disease incidence (%) = Number of infected tillers / Total number of observed tillers × 100.....(1)**

Disease severity should be estimated using a standardized rice sheath-blight rating scale. A disease severity index can then be calculated from the ratings assigned to individual plants or tillers. The relative vertical progress of disease within the canopy should also be recorded because sheath blight develops progressively from lower sheaths towards upper portions of the plant. The Area Under Disease Progress Curve (AUDPC) should be calculated to integrate disease development over time as equation 2-

$$AUDPC = \Sigma [(Y_i + Y_{i+1})/2] \times (t_{i+1} - t_i) \quad \textbf{.....(2)}$$

where Y represents disease severity and t represents the corresponding observation time. AUDPC will be particularly valuable for relating cumulative disease development to rhizosphere biomarker trajectories.

**2.10 Canopy microclimate monitoring**

Because the nitrogen-sheath blight relationship is strongly influenced by canopy conditions, measurements of canopy microclimate should be incorporated into the experiment. Temperature and relative humidity sensors can be installed at representative canopy heights.

Where facilities permit, the following parameters can also be monitored:

I. Canopy temperature
II. Relative humidity
III. Duration of high-humidity periods
IV. Leaf/sheath wetness duration
V. Solar radiation
VI. Soil temperature
VII. Soil moisture
VIII. Water depth

These observations will help determine whether excessive nitrogen increases disease partly by modifying canopy structure and microclimate.

**2.11 Nitrogen-use efficiency**

Nitrogen-use efficiency should be calculated using yield and nitrogen uptake data. Agronomic nitrogen-use efficiency can be estimated as equation 3-

**Agronomic NUE = (Yield in fertilized treatment − Yield in unfertilized control) / Amount of N applied.....(3)**

Partial factor productivity can also be calculated as equation 4-

**PFP-N = Grain yield / Amount of N applied.....(4)**

Nitrogen recovery efficiency and physiological efficiency may also be calculated where complete nitrogen uptake measurements are available. The biomarker-guided treatment will be considered successful only if it reduces unnecessary nitrogen input while maintaining acceptable yield and improving or maintaining nitrogen-use efficiency.

**2.12 Biomarker-guided nitrogen management**

The critical experimental component will be the development and testing of a decision rule based on the biomarker panel. For example, nitrogen application could be recommended when crop nitrogen status indicates developing deficiency and soil mineral nitrogen is below a predefined threshold. Nitrogen application could be postponed when crop nitrogen status is adequate and rhizosphere mineral nitrogen is already high. If high nitrogen status coincides with high pathogen abundance and increasing disease risk, further nitrogen should be avoided and integrated sheath-blight management should be initiated. Importantly, these thresholds should not be arbitrarily selected. They should first be developed from the factorial experiment and subsequently validated in independent field trials.

**2.13 Integration of biomarkers into a disease-risk index**

The individual biomarkers can be standardized and integrated into a composite index. Candidate variables may include:

Nitrogen Risk Component: mineral N + plant N + microbial N balance
Rhizosphere Health Component: microbial biomass + enzyme activity + microbial diversity
Pathogen Component: R. solani abundance + disease incidence
Environmental Component: soil moisture + canopy humidity + temperature
Host Component: Canopy density + leaf nitrogen + chlorophyll status

Each biomarker can initially be standardized using z-scores or another appropriate statistical transformation. Multivariate statistical techniques can then be used to determine which biomarkers provide the strongest predictive

value. The resulting index may be expressed as a low-, moderate- or high-risk category for nitrogen-associated sheath blight development.

**2.14 Statistical analysis**

Data should first be tested for normality and homogeneity of variance. Analysis of variance should be performed using an appropriate mixed-effects or factorial model, with nitrogen, irrigation and their interaction treated as fixed effects and blocks or other appropriate experimental factors treated as random effects. Repeated measurements of biomarkers and disease variables should be analysed using repeated-measures or mixed-effects models.

Correlation analysis can initially identify relationships among:

I. Mineral nitrogen and disease severity
II. Plant nitrogen and sheath blight
III. Microbial biomass and pathogen abundance
IV. Pathogen abundance and AUDPC
V. Canopy humidity and disease severity
VI. Rhizosphere enzyme activity and nitrogen-use efficiency

Principal component analysis can be used to identify major biomarker combinations. Regression, partial least-squares regression, random forest or other suitable machine-learning methods can then be used to identify the most predictive biomarker combinations. The predictive model should be evaluated using independent validation data rather than only the data used to develop the model. Measures such as coefficient of determination, root mean square error, sensitivity, specificity and area under the receiver operating characteristic curve can be used where appropriate.

**2.15 Microbiome and molecular analysis**

A subset of samples representing contrasting treatments and disease states should be selected for high-throughput microbial community analysis. Bacterial and fungal community profiles can be compared among low-N, recommended-N, high-N and biomarker-guided treatments. Particular attention should be given to whether high nitrogen and intensive irrigation cause reproducible changes in microbial community structure associated with increased sheath blight risk. Network analysis may be used to identify microbial taxa that consistently occur with either pathogen abundance or disease suppression. Quantitative PCR measurements of R. solani should be correlated with conventional disease observations to determine whether molecular pathogen abundance can function as an early-warning biomarker.

**2.16 Validation experiment**

Following the initial screening experiment, the most informative biomarkers should be selected to create a simplified field-level panel. A second independent experiment should then compare:

Conventional farmer/recommended practice versus biomarker-guided nitrogen and disease management. The validation experiment should be conducted over at least two seasons and, preferably, at multiple locations representing contrasting soil and irrigation conditions. The principal criteria for success should include reduced nitrogen input, lower sheath blight severity or AUDPC, improved nitrogen-use efficiency, maintenance or improvement of grain yield, and economic advantage.

**2.17 Economic analysis**

The economic feasibility of the biomarker approach should be evaluated by comparing the cost of soil, plant, and molecular analyses with savings resulting from reduced nitrogen and fungicide use and any increase in grain yield. The following parameters can be calculated:

I. Cost of cultivation
II. Cost of nitrogen fertilizer
III. Cost of disease-management inputs
IV. Cost of biomarker testing
V. Gross returns
VI. Net returns
VII. Benefit–cost ratio
VIII. Cost saved per hectare
IX. Additional income generated per unit of nitrogen saved

This analysis is essential because a technically accurate biomarker system will have limited practical value if its implementation cost is greater than the economic benefit obtained by farmers.

## 3. RESULTS AND DISCUSSION

**3.1 Rhizosphere biomarker response to nitrogen and irrigation management**

The experimental framework demonstrated that the nitrogen–sheath blight relationship in intensively irrigated paddy should be interpreted as a combined soil, rhizosphere, plant and pathogen phenomenon rather than as an isolated disease problem. The biomarker panel provided a multidimensional approach for assessing nitrogen availability, biological nitrogen cycling, pathogen pressure, beneficial microbial activity and the disease-suppressive capacity of the rhizosphere. This approach is particularly relevant to irrigated rice because nitrogen-use efficiency is generally low, with the biomarker document indicating that only about 30-40% of applied nitrogen is typically recovered by the crop, while the remainder may be lost through nitrification–denitrification

and volatilization. The results should therefore be interpreted in terms of the balance between nitrogen supplied, nitrogen retained within the rhizosphere, nitrogen taken up by the rice plant, and nitrogen potentially lost from the system. The biomarker panel makes it possible to distinguish a biologically active rhizosphere capable of retaining and recycling nitrogen from a system in which large quantities of applied nitrogen remain vulnerable to loss. This distinction is important because simply increasing nitrogen supply may increase vegetative growth without proportionately increasing grain production and may simultaneously create conditions favourable for sheath blight.

**3.2 Rhizoctonia solani as the principal Disease-Risk Biomarker (DRB)**

Among the pathogen biomarkers evaluated, Rhizoctonia solani emerged as the most important target for the proposed rice disease-risk radar. The biomarker document identifies R. solani as the principal marker associated with sheath blight and notes that its sclerotia survive in soil and straw. Pre-season soil and residue inoculum therefore provide an important indication of subsequent disease risk, particularly when combined with high nitrogen and dense crop canopies. The expected relationship between pathogen abundance and disease development is not necessarily linear. Detection of R. solani in soil indicates inoculum pressure, but epidemic development depends on the interaction between pathogen inoculum, host susceptibility, canopy density, humidity, irrigation conditions and plant nutritional status. Consequently, quantitative pathogen detection should be interpreted together with nitrogen and environmental biomarkers rather than used as an independent disease forecast.A progressive increase in R. solani abundance from the vegetative stage towards maximum tillering and heading would be expected to precede or accompany increasing sheath-blight incidence and severity. If molecular pathogen measurements increase before visible symptoms, this would demonstrate the principal practical advantage of the biomarker approach: disease risk could potentially be identified before the crop reaches a stage at which extensive sheath infection is already established.

**3.3 Nitrogen availability and the nitrogen-sheath blight relationship**

The nitrogen-related biomarkers indicate that the critical issue is not nitrogen deficiency alone but the synchronization between nitrogen availability and crop demand. The panel includes markers associated with nitrogen availability, biological nitrogen fixation, and ammonium–nitrate transformation. In intensively fertilized paddy, high mineral nitrogen accompanied by adequate or excessive plant nitrogen status would indicate a potential nitrogen-surplus condition. Such a condition could favour excessive vegetative development and increase canopy density. Dense canopies can retain humidity and prolong the favourable microenvironment for sheath blight development. Thus, a positive association between high mineral nitrogen, elevated plant nitrogen status, dense canopy development and increasing R. solani abundance would provide biological evidence for the proposed nitrogen–sheath blight loop. An important feature of the rice system is that nitrogen biomarkers cannot be interpreted in exactly the same manner as in aerobic upland crops. The biomarker document emphasizes that nitrification-related markers should be interpreted inversely under continuous flooding. Rice can directly utilize ammonium, whereas nitrate produced in the oxidized soil layer may subsequently move into reduced zones and be lost through denitrification. Therefore, a strong nitrification signal under continuously flooded conditions may represent nitrogen-loss risk rather than improved nitrogen fertility. Under drained, alternate wetting and drying or aerobic conditions, however, the same signal can have a different interpretation. This observation is important for developing a rice-specific biomarker algorithm. A universal interpretation of nitrogen-cycling biomarkers would be inappropriate for paddy soils because flooding changes the redox environment and consequently changes the direction and magnitude of several nitrogen transformations.

**3.4 Rhizosphere microbial activity and disease suppressiveness**

The results framework also indicates that the biological component of the rhizosphere is central to breaking the nitrogen–sheath blight loop. Beneficial microorganisms such as Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus velezensis, Trichoderma harzianum and Trichoderma asperellum have potential roles in pathogen suppression and plant growth promotion. The biomarker document particularly identifies B. subtilis as an important antagonist of soil-borne pathogens and associates high counts with genuine suppressiveness against R. solani. The expected outcome is that rhizospheres with greater populations or activity of beneficial organisms would show reduced pathogen establishment or slower disease development compared with biologically weaker rhizospheres. However, the results should be interpreted in relation to the irrigation regime. The document emphasizes that aerobic biocontrol organisms are most active in the nursery, oxidized surface soil, the oxygenated rhizosphere and during drainage or alternate wetting and drying, whereas their activity may decline in continuously submerged bulk soil. This provides a mechanistic explanation for why irrigation management may influence biological sheath-blight suppression. A carefully managed wetting and drying cycle may create short periods that favour beneficial aerobic microorganisms while simultaneously improving nitrogen-use efficiency. Thus, water management can potentially influence sheath blight indirectly by modifying both nitrogen transformations and rhizosphere biological activity.

**3.5 Functional biomarkers of sheath-blight suppression**

Among the functional biomarkers, BS217 and BS265 are particularly important for the proposed disease-suppressiveness assessment. BS217 represents functional capacity for destabilizing fungal cell walls, while BS265 is associated with disruption of fungal cellular metabolism. The biomarker document identifies BS217 as a particularly valuable marker for assessing the soil's chitinolytic capacity against Rhizoctonia sclerotia and Fusarium in crop residues, while BS217 and BS265 together can form a practical sheath-blight suppressiveness

index.An increase in these functional signals accompanied by lower R. solani abundance and reduced AUDPC would provide stronger evidence of biological suppression than measurements of beneficial microbial abundance alone. This distinction is important because the mere presence of an antagonist does not necessarily indicate that it is functionally active. Functional biomarkers provide information closer to the biological process responsible for pathogen suppression.Therefore, the most informative result would be a negative relationship between the disease-suppressiveness index and R. solani abundance or disease progress. Such a relationship would support the use of functional biomarkers as components of a predictive sheath-blight management system.

**3.6 Nitrogen fixation and reduced dependence on fertilizer nitrogen**

The nitrogen-fixation biomarkers also provide an important dimension to the proposed system. The panel includes markers associated with atmospheric nitrogen fixation and regulation of nitrogen-fixation activity. The document notes that biological nitrogen fixation has historically contributed to nitrogen supply in paddy systems and that a strong nitrogen-fixation signal may provide justification for reducing basal urea inputs. The significance of this finding lies in shifting the nitrogen-management objective from maximizing fertilizer input to maximizing total biologically available nitrogen. If strong biological nitrogen-fixation activity coincides with adequate plant nitrogen status, additional basal fertilizer nitrogen may provide diminishing returns. Biomarker-guided nitrogen management could therefore reduce the amount of readily available nitrogen that contributes to excessive canopy development while maintaining an adequate nitrogen supply for crop growth. Such a strategy could potentially weaken the first component of the nitrogen–sheath blight loop: excessive nitrogen availability.

**3.7 Straw decomposition, carbon cycling and nitrogen immobilization**

Rice straw represents another important component of the rhizosphere system. The biomarker document identifies B43 and BS065 as indicators of structural and non-structural sugar degradation and soil-carbon recycling. Their practical importance arises from the large, silica-rich straw load generated by rice production. Weak activity of these markers may indicate inadequate residue decomposition and possible accumulation of straw-associated material that can immobilize nitrogen. The relationship between residue decomposition and sheath blight is particularly important because R. solani sclerotia can persist in soil and crop residues. Consequently, efficient residue turnover combined with biological antagonism may reduce the persistence of pathogen inoculum while improving nutrient cycling.A rhizosphere showing strong residue-decomposition activity, adequate microbial biomass, and high disease-suppressive functional activity would therefore be expected to have greater biological resilience than a system characterized by slow residue turnover and weak suppressive activity. This suggests that straw management should not be considered independently of disease management. Instead of treating rice residues simply as waste, the biomarker approach considers residue decomposition as a component of nutrient cycling and pathogen-risk management.

**3.8 Interaction between irrigation, nitrogen cycling and disease risk**

The interaction between irrigation and nitrogen was one of the most important conceptual outcomes of the biomarker framework. Flooding changes the redox state of the soil and consequently alters nitrogen transformation, phosphorus availability, microbial activity and root physiology. The biomarker document specifically notes that nitrogen-cycling markers require different interpretation under flooded and drained conditions. Under intensive continuous irrigation, high nitrogen availability combined with dense vegetation may increase the risk of sheath blight. In contrast, appropriately managed alternate wetting and drying can introduce aerobic phases into the rhizosphere, potentially modifying microbial activity and nitrogen transformations. The document also identifies BS080 as a water-stress tolerance marker relevant to rice under alternate wetting and drying and BS008 as a stress-tolerance marker associated with ethylene regulation under both flooding and drought conditions.Therefore, the biomarker response should be interpreted as a dynamic response to the wetting–drying history of the soil rather than as a static soil property.

**3.9 Development of a rhizosphere disease-risk profile**

The combined biomarker information can be used to construct a rhizosphere disease-risk profile for each field. A high-risk profile would be characterized by high available nitrogen, high plant nitrogen status, strong vegetative growth, high R. solani abundance and weak disease-suppressive functional activity. A low-risk profile would be expected to show balanced nitrogen availability, efficient nitrogen cycling, strong beneficial microbial activity, lower pathogen pressure and higher functional suppressiveness. This integrated interpretation is more informative than any individual measurement. For example, a field may contain detectable R. solani but remain at relatively low disease risk if pathogen abundance is accompanied by strong biological suppression and balanced nitrogen management. Conversely, moderate pathogen abundance may become highly consequential when combined with excessive nitrogen, dense canopy development and weak rhizosphere suppressiveness. Thus, the proposed biomarker panel should ultimately be converted into a composite risk index rather than used as a simple list of independent measurements.

**3.10 Proposed mechanism for breaking the nitrogen–sheath blight loop**

The results collectively support a conceptual model in which the nitrogen–sheath blight loop can be interrupted at multiple points.

Excessive nitrogen → excessive vegetative growth → dense and humid canopy → favourable sheath-blight environment → increasing R. solani activity → disease development → yield loss → further nitrogen application. The biomarker-guided strategy attempts to interrupt this sequence through several mechanisms. Nitrogen biomarkers identify surplus nitrogen and prevent unnecessary fertilizer application. Plant biomarkers identify

excessive crop nitrogen status. Pathogen biomarkers provide early warning of R. solani pressure. Functional microbial biomarkers identify the biological suppressiveness of the rhizosphere. Irrigation biomarkers and soil-redox indicators help determine whether water management is promoting nitrogen loss or supporting beneficial microbial processes. The strongest evidence for successful disruption of the loop would therefore be obtained when biomarker-guided management simultaneously produces lower nitrogen input, improved nitrogen-use efficiency, reduced R. solani abundance or disease progress, stronger rhizosphere suppressiveness, and stable or increased grain yield.

**3.11 Implications for integrated rice disease and nutrient management**

The findings have implications beyond sheath blight. The uploaded biomarker framework identifies a broader disease-risk radar including Fusarium, Pythium, Xanthomonas, Alternaria and plant-parasitic nematodes, although R. solani remains the highest-priority target for the present nitrogen–sheath blight study.The same rhizosphere platform could therefore eventually be adapted for integrated rice health management. For example, Xanthomonas detection could provide an early indication of bacterial disease risk, while Meloidogyne and Pratylenchus markers could support root-health assessment. However, these secondary biomarkers should not dilute the primary objective of the present study, which is to resolve the interaction among nitrogen supply, rhizosphere biological functioning and sheath blight.

**4. Practical significance and implications**

The principal practical value of the biomarker approach will be moving rice management from fixed fertilizer schedules and symptom-based disease control towards field-specific decision making. Instead of applying nitrogen according to calendar dates alone, farmers could use a combination of crop nitrogen status, soil nitrogen availability and biological indicators to determine whether additional nitrogen is justified. Similarly, instead of waiting for severe sheath-blight symptoms, pathogen and functional suppressiveness biomarkers could identify fields entering a high-risk phase. This would permit earlier and more targeted interventions. The approach is particularly attractive for intensively irrigated rice because the same management decisions that influence nitrogen-use efficiency also influence rhizosphere oxygen status, microbial activity and disease development. Consequently, nitrogen and disease management should not be treated as separate programmes.

**4.1 Paddy disease and soil-health context**

The dominant biotic threats in paddy are sheath blight and the associated sheath/stem rot complex, blast, bacterial leaf blight, brown spot and false smut on the foliage and panicle, bakanae and seedling rots in the nursery, and plant-parasitic nematodes, particularly rice root-knot nematode in direct-seeded and aerobic rice and rice root nematodes in flooded fields. On the soil side, the defining constraints are nitrogen-use efficiency (typically only 30-40% of applied N is recovered, the rest lost to nitrification–denitrification and volatilization), organic-matter turnover of the large straw residue load, zinc and iron dynamics under reduction, and increasingly grain contamination by arsenic and cadmium mobilized under flooded or alternately flooded conditions. The panel below is organized against these priorities.

**4.1.1 Pathogenic Biomarkers Relevant to Paddy (Disease Risk Radar)**

**I.High-priority pathogen markers**

These markers map onto the most economically damaging paddy diseases and should form the core of routine monitoring in nursery beds, at maximum tillering, and post-harvest in the residue layer.

| Marker / Species | Paddy Disease Association | Why It Matters for Paddy |
|---|---|---|
| Rhizoctonia solani | Sheath blight; also aggregate sheath spot and banded leaf blight | The single most important marker in this panel for rice. Sheath blight is the top yield-limiting disease of intensively fertilized paddy, and R. solani sclerotia overwinter in soil and straw. Pre-season soil and residue loads predict outbreak severity under dense canopies and high N. |
| Fusarium spp. / F. oxysporum / F. solani | Bakanae (foot rot), seedling blight, root and crown rot | Bakanae is seed- and soil-borne and causes stand loss and sterile panicles; Fusarium signal in nursery beds and seed-bed soil is an early warning to treat seed and rogue affected seedlings. |
| Pythium aphanidermatum | Seedling damping-off, water rot, root rot | Classic problem of wet nursery beds and direct-seeded rice under saturated soil; a leading cause of poor stand establishment. |
| Sclerotium rolfsii | Stem rot / sclerotial rot complex | Closest available library proxy for the sclerotial stem-rot pathogens of rice, which are strongly residue-borne and increase under continuous rice–rice cropping. |

| Marker / Species | Paddy Disease Association | Why It Matters for Paddy |
|---|---|---|
| Meloidogyne spp. / M. incognita | Root-knot nematodes (proxy for rice root-knot nematode, M. graminicola) | The dominant nematode threat in direct-seeded, aerobic, and rice–vegetable rotation systems; causes galling, hidden yield loss of 20–80% in severe cases, and worsens nutrient deficiency symptoms. |
| Pratylenchus spp. | Root-lesion nematodes (proxy for rice root nematodes in flooded soil) | Damages the root cortex, opens infection courts for Fusarium and Pythium, and is common in upland and rainfed lowland rice. |
| Xanthomonas spp. | Bacterial leaf blight and bacterial leaf streak (X. oryzae pathovars) | Bacterial leaf blight is a major epidemic disease in irrigated paddy; genus-level detection in residue, irrigation channels, or stubble is an early season alert. |
| Alternaria spp. | Foliar/panicle leaf-spot complex (proxy for brown spot and grain discoloration fungi) | Brown-spot-type diseases are indicators of nutritionally weak, silicon- and potassium-deficient soils; the marker doubles as a soil-fertility stress signal. |

**II. Secondary / situational pathogen markers**

Relevant mainly in rice-based rotations (rice–vegetable, rice–potato, rice–pulse), in upland rice, or on bunds and field margins.

| Marker / Species | Relevance in a Paddy System |
|---|---|
| Rotylenchulus reniformis | Reniform nematode; relevant where paddy rotates with cotton, pulses, or soybean, and it carries over to the rice phase. |
| Phytophthora capsici / cactorum / cinnamomi / fragariae | Low direct relevance to rice; monitor only where rice rotates with chillies, cucurbits, or solanaceous vegetables, which are highly susceptible in the dry season. |
| Sclerotinia sclerotiorum | Relevant to pulse and oilseed crops grown in the rice fallow; not a rice pathogen. |
| Verticillium dahliae / Pyrenochaeta spp. / Clavibacter michiganensis / Candidatus Liberibacter solanacearum | Rotation-crop pathogens (tomato, onion, potato, carrot). Retain only if those crops enter the sequence. |
| Neopestalotiopsis spp. / Armillaria spp. | Negligible relevance to paddy; perennial and berry-crop pathogens. |
| Paecilomyces lilacinus (listed in A.2) | Listed as a pathogen but functions as a nematode egg parasite — see Section 4, where it is treated as a beneficial for rice root-knot nematode management. |

**III. Beneficial biomarkers Relevant to Paddy (Bio-Stimulants & Bio-Controls)**

All ten beneficial markers apply, but their value differs sharply between the flooded and drained phases of the season. Aerobic bio-control agents are most active in the nursery, in the oxidized surface layer, in the rhizosphere oxygenated by rice aerenchyma, and during drainage or alternate wetting and drying.

| Marker / Species | Function | Paddy Application |
|---|---|---|
| Bacillus subtilis | Bio-control of soil-borne pathogens | The leading bacterial antagonist for sheath blight and seedling diseases in rice; tolerates the low-oxygen bulk soil better than fungal agents and colonizes the sheath and rhizoplane. High counts indicate genuine suppressiveness against R. solani. |
| Bacillus amyloliquefaciens | Root colonization, antibiotic production | Strong lipopeptide producer active against Rhizoctonia and Fusarium; also promotes tillering through growth-regulator activity. |

| Marker / Species | Function | Paddy Application |
|---|---|---|
| Bacillus velezensis IB32 / NC30 | Growth promotion, pathogen suppression | Well suited to rice as a seed and seedling-root treatment before transplanting; suppresses the sheath blight and bakanae complex. |
| Trichoderma harzianum | Bio-fungicide, root development | Highly effective in nursery beds, seed treatment, and straw-compost inoculation, where it both antagonizes R. solani sclerotia and accelerates residue decomposition. Activity falls in continuously submerged bulk soil, so target the aerobic windows. |
| Trichoderma asperellum | Bio-control, systemic resistance inducer | Primes rice defenses against blast and sheath blight; useful as a nursery drench before transplanting. |
| Bacillus megaterium | Phosphate solubilization | Valuable in the nursery and in aerobic/direct-seeded rice on acidic or calcareous soils. Note that flooding itself releases fixed P through iron reduction, so this marker matters most before submergence and in AWD systems. |
| Azotobacter vinelandii | Free-living nitrogen fixation | A marker of biological N input in the oxidized surface layer and rhizosphere. Given rice's poor recovery of applied N, biological fixation capacity is a direct efficiency indicator. (In fully anaerobic paddy, anaerobic and cyanobacterial fixers dominate — see Section 8.) |
| Streptomyces griseoviridis / Streptomyces K61 | Suppression of root rot, wilt, damping-off | Best deployed in nursery beds and seed-bed media where damping-off and seedling blight pressure is highest. |
| Paecilomyces lilacinus (listed in A.2) | Nematode egg parasite | Directly useful against rice root-knot nematode in direct-seeded and aerobic rice; one of the few practical biological levers against M. graminicola. |

**4.1.2 Functional Gene Markers Relevant to Paddy (Metabolic Pathways & Soil Health)**

The functional markers are crop-agnostic by design. The interpretation column below is where paddy diverges most from every other crop in the library, because submergence inverts the meaning of several nitrogen markers.

| Marker | Function (per library) | Paddy Interpretation |
|---|---|---|
| T22 | Nitrogen availability | Baseline N supply capacity. Rice removes large quantities of N per tonne of grain plus straw; this marker tracks whether the soil can sustain it between top-dressings. |
| BS114 | Atmospheric nitrogen fixation | Measures biological N input — significant in paddy, where free-living, associative, and cyanobacterial fixation historically sustained rice yields without fertilizer. A strong signal justifies reducing basal urea. |
| BS181 | Regulation of the key nitrogen-fixation enzyme | Confirms fixation genes are actively expressed and regulated, not merely present in the community. |
| BS020 / BS279 | Ammonium–nitrate conversion (nitrification/oxidation–reduction) | Read these inversely in flooded paddy. Rice takes up ammonium directly; nitrate formed in the oxidized layer is carried into the reduced zone and lost to denitrification. A high nitrification signal in a continuously flooded field is a warning of nitrogen loss, not a sign of fertility. In drained, AWD, or aerobic rice the same signal is positive. |
| B357 | Ammonium→nitrate conversion plus structural-sugar degradation for symbiosis and carbon recycling | Dual-purpose in paddy: the nitrification half carries the loss-risk caveat above, while the sugar-degradation half is highly relevant to the heavy straw residue load returned to the field. |
| BS019 | Phosphorus solubilization under scarcity | Most informative in nursery soil, aerobic rice, and AWD fields. Under continuous flooding, iron reduction releases occluded P on its own, so a weak signal is less alarming than it would be in an upland crop. |

**4.1.3 Stress tolerance and water relations**

| Marker | Function (per library) | Paddy Interpretation |
|---|---|---|
| BS080 | Water-stress tolerance via quaternary ammonium; organic-matter sensor | Critical for direct-seeded, aerobic, and rainfed lowland rice, and for any field under alternate wetting and drying, where the crop experiences real drought episodes between irrigations. |
| BS008 | Stress tolerance via reduction of ethylene levels | Unusually important in rice: ethylene accumulates under submergence and drives shoot elongation and lodging, while ACC-deaminase-type activity protects root growth under both flooding and drought stress in the same season. |
| BS214 | Water retention and nutrient availability via biofilm formation | Supports nutrient retention in coarse-textured, leaky paddy soils and stabilizes the rhizosphere during wetting–drying cycles. |

**4.1.4 Disease-suppressiveness capacity**

| Marker | Function (per library) | Paddy Interpretation |
|---|---|---|
| BS217 | Destabilization of fungal cell walls | A direct measure of the soil's chitinolytic firepower against Rhizoctonia sclerotia and Fusarium in residue — the highest-value functional marker for sheath blight risk. |
| BS265 | Disruption of fungal cellular metabolism | Complements BS217; together they form a practical sheath-blight suppressiveness index for the field. |
| B3 | Bactericidal against Xanthomonas, Erwinia, Pseudomonas, Clavibacter | Explicitly names Xanthomonas — the bacterial leaf blight genus. This is the key functional marker for bacterial disease suppression in irrigated paddy. |
| BS002 | Resistance to compounds secreted by phytopathogenic bacteria | Indicates that the beneficial community can persist under bacterial pathogen pressure rather than being displaced by it. |

**4.1.5 Carbon, residue turnover, and remediation**

| Marker | Function (per library) | Paddy Interpretation |
|---|---|---|
| B43 / BS065 | Structural and non-structural sugar degradation; soil carbon recycling | The most practically useful pair for paddy residue management. Rice straw is bulky, silica-rich, and slow to break down; these markers show whether incorporated straw is actually being mineralized or is merely accumulating and immobilizing nitrogen. Weak signals are a direct argument for decomposer inoculation instead of straw burning. |
| BS089 | Atmospheric CO2 sequestration (carbon mapping) | Supports soil-carbon and sustainability reporting. Note that for paddy the dominant greenhouse gas is methane, which this marker does not capture — see Section 8. |
| BS202 | Sequestration of heavy metals (cadmium, zinc, cobalt) | High-value for rice food safety. Cadmium is mobilized in aerobic and AWD paddy soils and accumulates in grain; cadmium-sequestering microbial capacity is directly relevant to grain compliance limits. Zinc relevance is dual-edged — zinc deficiency is the most widespread micronutrient disorder in flooded rice. |
| BS024 | Degradation of chlorohexane and chlorobenzene | Situational: relevant in intensively sprayed rice tracts and in fields with a legacy of organochlorine use. |

## 5. CONCLUSION

The biomarker framework indicates that sheath blight in intensively irrigated paddy is best understood as an emergent outcome of interactions among nitrogen availability, irrigation-induced soil conditions, crop canopy development, pathogen inoculum and rhizosphere biological activity. Rhizoctonia solani represents the central pathogen biomarker, while nitrogen-cycling, beneficial-microbial and fungal-suppressiveness markers provide

complementary information about the conditions that determine whether pathogen presence develops into economically important disease. The uploaded framework specifically identifies BS217 and BS265 as promising functional indicators for a sheath-blight suppressiveness index and highlights the importance of nitrogen-cycling markers in distinguishing efficient nitrogen retention from potential nitrogen loss under flooded rice conditions. The proposed results therefore support a shift from a single-variable disease-management model to an integrated Rhizosphere Biomarker–Nitrogen–Disease Risk System. Its success should ultimately be demonstrated through independent field validation showing that biomarker-guided nitrogen and irrigation decisions reduce nitrogen losses and sheath-blight pressure while maintaining yield and improving the economic efficiency of rice production. Because the present source document does not provide measured treatment data, these conclusions should be treated as the biological interpretation and expected experimental outcome framework; actual numerical means, statistical significance, correlations and effect sizes should be inserted only after the field and laboratory observations are available. In the future, the rhizosphere biomarker panel could become an important component of climate-smart and resource-efficient rice production. Its greatest value will not lie in producing more measurements, but in converting biological information into timely management decisions. The central principle is simple: instead of asking only how much nitrogen the rice crop has received or whether sheath blight is already visible, the farmer should ask what the rhizosphere is indicating about the balance among nitrogen supply, plant demand, microbial functioning, pathogen pressure and environmental conditions. When these signals are interpreted together, the nitrogen–sheath blight loop can be interrupted before it becomes a self-reinforcing cycle. This offers a pathway towards rice production systems that maintain high productivity while using nitrogen and water more efficiently and relying increasingly on the biological capacity of the rhizosphere to support crop health. One of the most important consequences of this management combination is the development of a nitrogen–sheath blight loop in which abundant nitrogen promotes luxuriant vegetative growth and a humid canopy environment, while severe sheath blight reduces photosynthetic efficiency and grain yield, encouraging farmers to compensate with still higher fertilizer inputs. Sheath blight, primarily caused by Rhizoctonia solani, is particularly important in intensively cultivated rice because the pathogen survives in soil and crop residues and spreads rapidly through plant-to-plant contact under warm, humid, and continuously wet conditions. Breaking this cycle requires a shift from reactive disease management towards an integrated understanding of the soil–root–microbe–plant system.

**Declaration of competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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