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Metabolic fingerprints of rhizosphere microbial communities differ due to increased nitrogen availability and cultivation methods of rice (*Oryza sativa*)

SAGAR SANKIHALLY PUTTAPPA[1], BABANPREET KOUR[1,2] and BALASUBRAMANIAN RAMAKRISHNAN[1]*

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

Microbial metabolic activities in the rhizosphere are essential for plant nutrition and yield. The study was carried out during 2023–2024 at ICAR-Indian Agricultural Research Institute, New Delhi to profile the rhizosphere metabolic community fingerprints using Biolog EcoPlate™ assays in rice (*Oryza sativa* L.) grown on neutral soil under conventional flooded (CF) and aerobic [simulating direct-seeded rice (DSR)] methods, with high (HN, 150 kg N/ha) or low (LN, 25 kg N/ha) nitrogen (N). The experiment was laid out in a randomised block design (RBD). Average well colour development (AWCD) of total and class-specific substrates, diversity indices, and principal component analysis (PCA) were used to assess community-level metabolic activity, diversity, and carbon source utilisation patterns (CSUP). Low-N treatments (CFLN, DSRLN) showed higher AWCD (1.45 and 1.31, respectively) and broader substrate utilisation, indicating enhanced microbial activity and metabolic flexibility. The low-N treatment under DSR (DSRLN) had the most metabolically diverse and even communities, whereas the CF system (CFLN) favoured heterotrophic, oligotrophic communities adapted to anoxia. CSUP revealed differential catabolism of amino and carboxylic acids, with marginal variation in amine and polymer utilisation. PCA separated the CSUP along nitrogen and cultivation methods, high-N CF method (CFHN) favoured copiotrophic degradation of aromatics and polymers, while low-N DSR (DSRLN) promoted oxidative metabolism of keto acids, benzoates, and disaccharides. Functional indices reflected niche-specific adaptation, with DSRLN exhibiting the highest Shannon diversity and CFLN dominated by specialists for distinct carbon classes. Nitrogen availability and cultivation methods act as filters shaping rhizosphere metabolic activity, flexibility, and community stability, influencing sustainable rice cultivation. Therefore, understanding the management of rhizosphere microbiome under DSR is critical to unlock its potential as a sustainable, water-saving alternative to conventional flooded rice.

Keywords: Aerobic cultivation, Carbon utilisation, Conventional flooding, Microbial community, Nitrogen, Rice rhizosphere

Soil microbial communities are pivotal regulators of carbon turnover, nitrogen (N) cycling, and rhizosphere functionality in agricultural ecosystems (Philippot *et al.* 2013). These communities respond rapidly to chemical or physical disturbances. Their functional shifts often precede broader ecosystem changes, making them sensitive regulators of environmental change and early signals of ecological health and resilience. However, their metabolic versatility enables dynamic adaptation to environmental gradients, nutrient fluxes, and cultivation practices (Delgado-Baquerizo 2016). Rice (*Oryza sativa* L.), as a major staple cereal crop feeding over half the global human population, depends on rhizosphere microbial activities for nutrient acquisition, stress tolerance, and productivity (Misu *et al.* 2025). Agronomic interventions, such as N fertilisation and cultivation methods, can shift rhizosphere microbial community activities and carbon utilisation patterns, with N availability imposing selective pressures that favour functionally distinct taxa with specific resource-acquisition strategies (Chen *et al.* 2019). Generally, the conventional flooded rice (transplanted) produces higher yields than the direct-seeded rice (DSR), which also suffers from lower grain quality. Despite extensive work on taxonomic shifts and bulk enzymatic activities, fine-scale characterisation of how communities adapt metabolically to diverse C sources under varying N and cultivation methods remains poorly understood.

Community-Level Physiological Profiling (CLPP), assayed using the Biolog EcoPlate™, offers a sensitive means to characterise the community metabolism for multiple carbon substrates and generate metabolic fingerprints

[1]ICAR-Indian Agricultural Research Institute, New Delhi; [2]Monash University, Clayton, Melbourne. *Corresponding author email: ramakrishnanbala@yahoo.com

(Garland 1997). Each plate, containing 31 carbon sources (spanning amines/amides, amino acids, carbohydrates, carboxylic acids, polymers, and miscellaneous compounds) in triplicate with a tetrazolium redox dye, turns purple upon substrate oxidation, directly reflecting microbial metabolic activity. CLPP characterises communities along three axes, pattern similarity (functional resemblance), activity rate (colour-development kinetics), and functional richness (number of utilized substrates), with derived indices such as average well colour development (AWCD), Shannon-Wiener diversity index (H'), Shannon evenness index (E), Simpson diversity index (D) and McIntosh index (U) providing holistic views of metabolic breadth, dominance structures, and functional balance, while Bray-Curtis and Jaccard distances further capture spatial and temporal variability (Classen 2003). In parallel, carbon-source utilisation patterns (CSUP) serve as a tractable proxy for metabolic flexibility, linking broader substrate use with enhanced nutrient turnover, soil fertility, and plant productivity, and thereby guiding decisions on fertiliser inputs, soil management, and remediation. Its chief advantage is the rapid functional screening of complex carbon utilisation patterns of communities, thereby obviating the need for species identification. Although phenotype-based and sensitive to inoculum density and incubation conditions, CLPP can be a rapid, informative early-warning tool for tracking environmental and anthropogenic impacts on ecosystem processes. Importantly, these metabolic fingerprints can be more specific compared to soil attributes, such as *p*H, organic C, total N, microbial biomass, and metabolic profiles, which continue to serve as integrative indicators of soil quality and ecological resilience (Rutigliano *et al.* 2023).

The present study hypothesised that N availability and cultivation methods regulate microbial C metabolism characteristically by modulating substrate affinity, functional diversity, and metabolic flexibility in rice rhizospheres. Indiscriminate nitrogen use in agriculture can alter microbial metabolic processes, thereby influencing soil ecology and ultimately undermining its long-term health. To test this, the metabolic activities of rhizosphere microbial communities were profiled using Biolog EcoPlat^{TM} assays, capturing the dynamics of C-substrate utilisation, shifts in functional diversity, and class-specific metabolic adaptations (i.e. CSUP). This functional profiling was intended to inform strategies for optimising rhizosphere health, improving nutrient-use efficiency, and promoting sustainable rice intensification under environmental constraints.

MATERIALS AND METHODS

Experimental condition: The experiment was conducted during 2023–24 at the National Phytotron Facility, ICAR-Indian Agricultural Research Institute (32º05'53"N, 77º08'09"E; at an elevation of 1462 m amsl), New Delhi under controlled conditions in the glasshouse. The neutral alluvial soil was characterised as sandy clay loam with a *p*H of 7.81, electrical conductivity of 0.58 dS/m, organic C content of 5.82 g/kg, and available N and P of 83.91 and 8.22 mg/kg, respectively. The soil samples were placed in 10 kg soil capacity pots in triplicate, and the rice cultivar (cv.) Pusa Basmati 1509 was chosen for the study. The experiment was laid out in a randomised block design (RBD) under two conditions, conventional flooded (CF) and aerobic [simulating direct-seeded rice (DSR)] methods of cultivation. The treatments of high and low N were, High N in CF conditions (CFHN) (T_1); Low N in CF conditions (CFLN) (T_2); High N in DSR (DSRHN) (T_3); and Low N in DSR conditions (DSRLN) (T_4). High-N and low-N included fertiliser combinations of 150:75:75 NPK kg/ha (i.e. 66.7, 33.3, 33.3 NPK mg/kg soil) and 25:12;12 NPK kg/ha (i.e. 11.1, 5.3, 5.3 NPK mg/kg soil), respectively. Under the CF conditions, 20-day-old seedlings were transplanted into puddled soil, with the water-to-soil ratio maintained at 1.25:1 (v/v), while a 60% moisture level was sustained under the DSR conditions. The sensor-regulated day/night temperatures of 32°C/26°C, relative humidity of 90%, an 18 h photoperiod, and a photon flux density of 450 µmol/m^2/s system were maintained throughout the experiment period in the National Phytotron Facility. The rhizosphere soil samples were collected by uprooting the plants at the anthesis stage (60 days after transplantation in CF; 75 days after sowing in DSR).

Metabolic fingerprint analysis using the Biolog EcoPlate assays: The rhizosphere soil sample (25 g) was added to 225 mL of 0.85% physiological saline solution. The mixture was shaken at 150 rpm at 4°C for an hour, and each sample was serially diluted to a 10^{-3} dilution (Ge *et al.* 2018). The diluted samples (150 µL) were dispensed into Biolog EcoPlate^{TM} in triplicate and incubated at 28°C for 10 days. The readings from the Biolog EcoPlate^{TM} assay were recorded at every 24 h interval in the Biolog MicroStation^{TM} system (Biolog, Inc., Hayward, California, USA).

Evaluation of average well colour development (AWCD): Each well of the Biolog EcoPlate™ contains a distinct C substrate, and microbial metabolism within the wells reduces tetrazolium dye to formazan, producing a colourimetric change proportional to metabolic activity. The extent of colour development, indicating substrate utilisation (Garland 1997), is quantified by measuring absorbance at 590 nm and calculating the AWCD using the formula:

$$AWCD = \sum_{i=1}^{n}(C_i - R)/n \quad (1)$$

Where C_i and R represent the absorbance values of the substrate well and control well, respectively, and n is the total number of wells. Values of (C_i-R) less than 0.06 were considered zero to minimise background interference (Classen 2003, Janniche 2012). AWCD of all substrates ($AWCD_a$) and class-specific substrates [i.e. amines/amides ($AWCD_{am}$), amino acids ($AWCD_{ami}$), carbohydrates ($AWCD_c$), carboxylic acids ($AWCD_{ca}$), polymers ($AWCD_p$), and miscellaneous ($AWCD_m$] were calculated.

Determination of microbial metabolic functional diversity indices: Microbial metabolic diversity was evaluated using functional diversity indices, extending to

evenness parameters to characterise substrate utilisation patterns across the microbial community.

Shannon-Wiener Diversity Index (H′) quantifies microbial functional diversity by incorporating both the richness and relative abundance of substrate utilisation patterns (Janniche 2012):

$$H' = -\sum P_i \ln P_i \quad (2)$$

$$P_i = (C_i - R)/\sum(C_i - R) \quad (3)$$

In equations (2) and (3), P_i, Proportion of the absorbance value of the i^{th} well to the total absorbance across all wells and in equation (3) C_i and R represent the absorbance values of the substrate well and control well, respectively.

The probability that different microbial groups utilize two randomly selected substrates was measured through the Simpson Diversity Index (D) using equation (4), emphasizing dominance and evenness (Janniche 2012):

$$D = 1 - \sum P_i^2 \quad (4)$$

Shannon Evenness Index (E) evaluates the uniformity of substrate utilisation across the microbial community (Keylock 2005):

$$E = H'/\ln S \quad (5)$$

Where S, Number of substrates utilized.

The McIntosh Index (U) was used to assess microbial community diversity based on the vector distance of substrate utilisation frequencies, integrating both abundance and dominance to provide a sensitive measure of how metabolic activity is concentrated within dominant functional groups in the community (Zhu *et al.* 2020):

$$U = \sqrt{\sum (n_i)^2} \quad (6)$$

Where n_i, Relative absorbance value determined by deducting the absorbance value of the control well from each of the C source wells.

Statistical analysis: Principal component analysis (PCA) was performed on Day 8 (logarithmic growth phase) after normalizing absorbance values through equation (7) to relative substrate index (R_{si}) to mitigate bias from substrate class variability (Wang *et al.* 2007), calculated as:

$$R_{si} = (C_i - R)/AWCD \quad (7)$$

Statistical analyses, PCA plotting, and figure generation were executed using OriginPro® software (version 2025, OriginLab, Massachusetts, USA). As no significant variation ($p>0.05$) was observed using a two-way ANOVA with year (2023, 2024) and N × cultivation treatment (CFHN, CFLN, DSRHN, DSRLN) as fixed factors, including their interaction, for each response variable ($AWCD_a$, class-specific AWCD, functional diversity indices) between the two years, data collected from both years under controlled conditions were integrated and illustrated collectively in the tables and figures. Data in the time-series plot were expressed as mean ± standard deviation. Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was performed at a significance level of $p\leq0.05$.

RESULTS AND DISCUSSION

Metabolic activities of the rice rhizosphere microbial communities: The AWCD patterns (Fig. 1) showed an initial lag (day 1), followed by a sharp rise and stabilisation by day 9, indicating progressive substrate oxidation, stabilisation of metabolic activity and sustained heterotrophic respiration (Luo *et al.* 2016). Mean metabolic rate, reflected by the AWCD slope (days 1–10), was highest under low-N, CFLN (1.45) and DSRLN (1.31), compared to high-N treatments, CFHN (1.23) and DSRHN (1.19), signifying greater microbial respiration and functional capacity ($p<0.05$) under N limitation. Nitrogen scarcity likely promotes functional diversity, enabling diverse guilds to exploit multiple carbon sources, while likely increasing carbon-rich root exudation stimulates nutrient mobilisation through rhizosphere priming and enhanced C degradation, supporting growth and nutrient uptake by plants. Overall, N availability and cultivation method jointly shape microbial metabolic potential, corroborating previous evidence of N-driven restructuring of microbial communities and carbon cycling (Luo *et al.* 2023).

Comparative analysis of microbial metabolic diversity indices: Significant differences ($p<0.05$) were recorded across nitrogen levels and cultivation methods on the 8^{th} day of incubation (Table 1), marking the peak of microbial activity before the decline phase. The highest Shannon diversity in DSRLN (1.48) reflected greater substrate richness and evenness, followed by CFHN (1.45), DSRHN (1.41), and CFLN (1.31). Corresponding Shannon

Table 1 Metabolic diversity indices of the rice rhizosphere microbial communities as influenced by nitrogen availability and cultivation methods

Sample	Shannon-Wiener diversity index (H')	Shannon evenness index (E)	Simpson diversity index (D)	McIntosh index (U)
CFHN	1.447 ± 0.042^{ab}	0.970 ± 0.022^{a}	0.960 ± 0.032^{a}	7.291 ± 0.337^{c}
CFLN	1.310 ± 0.038^{c}	0.879 ± 0.020^{b}	0.944 ± 0.033^{a}	10.277 ± 0.475^{a}
DSRHN	1.409 ± 0.041^{b}	0.945 ± 0.022^{ab}	0.958 ± 0.030^{a}	7.297 ± 0.337^{c}
DSRLN	1.475 ± 0.043^{a}	0.989 ± 0.023^{a}	0.965 ± 0.033^{a}	7.586 ± 0.350^{b}
CD ($p\leq0.05$)	0.007	0.004	0.010	0.236

*Rhizosphere soil samples were from neutral *p*H soil. Indices were calculated on 8^{th} day of incubation. Treatment details are given under Materials and Methods.

evenness and Simpson trends indicated a metabolically versatile, resilient community in DSRLN, likely promoted by low-N input (Ju *et al.* 2024) and improved aeration under DSR conditions (Chunmei *et al.* 2020). Despite lower richness and evenness, CFLN showed the highest McIntosh index (10.28), indicating a relatively small subset of functional groups contributed disproportionately to total metabolic activity. This combination of the lowest Shannon diversity but highest McIntosh suggested a metabolically active yet compositionally constrained assemblage, in which respiration is focused on a narrow suite of carbon substrates. Ecologically, such specialisation may support efficient processing of selected C sources under low-N, anoxic conditions, but it also implies reduced functional redundancy and potentially greater sensitivity to shifts in substrate supply compared with the more even, diverse DSRLN communities. Elevated diversity in CFHN likely reflected facultative anaerobe activity in N-rich, reduced environments.

Carbon source metabolism patterns of rhizosphere microbial communities: The class-specific AWCD patterns revealed time-dependent metabolic activation across treatments (Fig. 2). Carboxylic acids consistently exhibited the highest AWCD, followed by amino acids and carbohydrates, reflecting microbial preference for readily oxidizable, energy-rich substrates. Amines and amides showed minimal utilisation, indicating weak enzymatic responsiveness or inefficient catabolism. DSRLN maintained the highest carboxylic acid activity ($AWCD_{ca}$, day 8), likely driven by elevated dehydrogenase activity and organic acid transport (Shimizu 2013). In contrast, DSRHN showed early suppression of $AWCD_{ca}$, probably due to N-induced inhibition of organic acid metabolism and dominance of copiotrophs with narrow substrate ranges (Zhang *et al.* 2024). Under flooded conditions, CFLN slightly exceeded CFHN, suggesting that mild N limitation under anoxia promotes carboxylic acid catabolism via fermentation or anaerobic respiration (Buckel 2021). Amino acid utilisation ($AWCD_{ami}$) peaked in CFLN on day 7, reflecting the use of amino acids as dual C and N sources under N deficiency, whereas CFHN showed the lowest values, possibly due to feedback inhibition or rhizospheric *p*H shifts (Chen *et al.* 2024). In DSR systems, DSRLN outperformed DSRHN throughout, suggesting aerobic activation of proteolytic pathways under oxidized, N-deficient conditions (Geisseler and Horwath 2008). As rice grains are intrinsically low in lysine, threonine, tryptophan, and methionine, such microbial amino acid utilisation may adversely influence grain development and maturity.

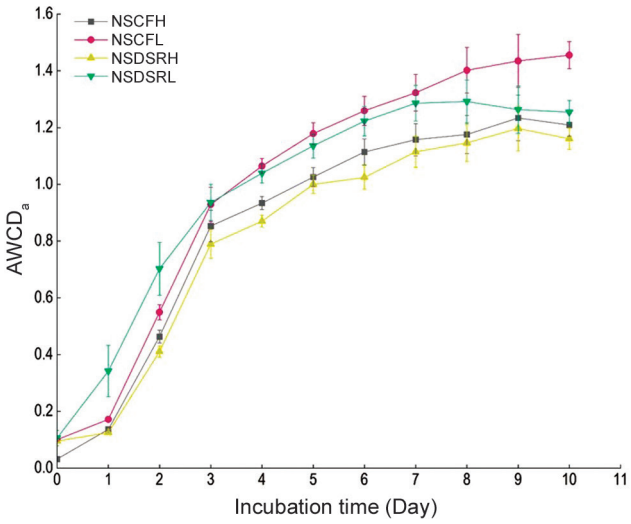

Fig. 1 The average well colour development of all ($AWCD_a$) carbon substrates of Biolog EcoPlates^{TM} by rice rhizosphere microbial communities with high and low N under two cultivation methods [Conventional flooding and DSR (aerobic)], during a 10-day incubation period.
Treatment details are given under Materials and Methods.

Among carbohydrates, CFLN recorded the highest $AWCD_c$ (day 7), implying intensified fermentative metabolism under N-deficient, anoxic conditions (Buckel 2021), whereas CFHN showed reduced utilisation likely due to N sufficiency favouring inorganic N-dependent taxa. In DSR, DSRLN surpassed DSRHN between days 3–8, indicating aerobic enhancement of carbohydrate catabolism through upregulated C-scavenging pathways utilizing root-exudate sugars (Mavrodi *et al.* 2021). CFHN exhibited the highest polymer utilisation ($AWCD_p$), consistent with increased hydrolase-mediated degradation under N-rich anaerobic conditions (Omura *et al.* 2024), while DSRLN showed limited polymer catabolism, reflecting constrained enzyme synthesis under N scarcity. DSRHN displayed maximum $AWCD_m$, denoting flexibility toward miscellaneous substrates, whereas DSRLN minimised $AWCD_m$ by conserving energy for core metabolism. For amines/amides, DSRHN maintained high $AWCD_{am}$ from day 4 onward, indicating ammonium-assimilating and nitrifying communities with active deaminase, amidase, and urease enzymes. Flooded systems exhibited lower $AWCD_{am}$ due to oxygen-limited microsites restricting aerobic amine degradation (Nguyen *et al.* 2008). Collectively, nitrogen availability and cultivation method synergistically governed CSUP patterns, with DSR under low N fostering broader metabolic engagement, stronger rhizosphere resilience, and enhanced C-N cycling efficiency.

Metabolic signatures reveal treatment-driven microbial functional heterogeneity: The treatment CFHN exhibited stronger catabolism of L-phenylalanine, D-xylose, D-galactonic acid, pyruvic acid methyl ester, L-arginine, phenylethylamine, tween 80, itaconic acid, 4-hydroxy benzoic acid, and 2-hydroxy benzoic acid (Fig. 3). This metabolic signature profile reflects high affinity for aromatics and polymers, directing active hydrolase-mediated degradation (Seo *et al.* 2009). Hydroxybenzoates and phenylethylamine utilisation suggest stress-responsive, facultative microbial strategies (Fu *et al.* 2015). Overall, CFHN supported versatile, copiotrophic microbial communities. The CFLN selectively utilized γ-aminobutyric acid, D-galacturonic acid, N-acetyl-D-glucosamine, i-erythritol, β-methyl-D-glucoside, β-hydroxy-glycyl-L-glutamic acid, L-serine, L-asparagine,

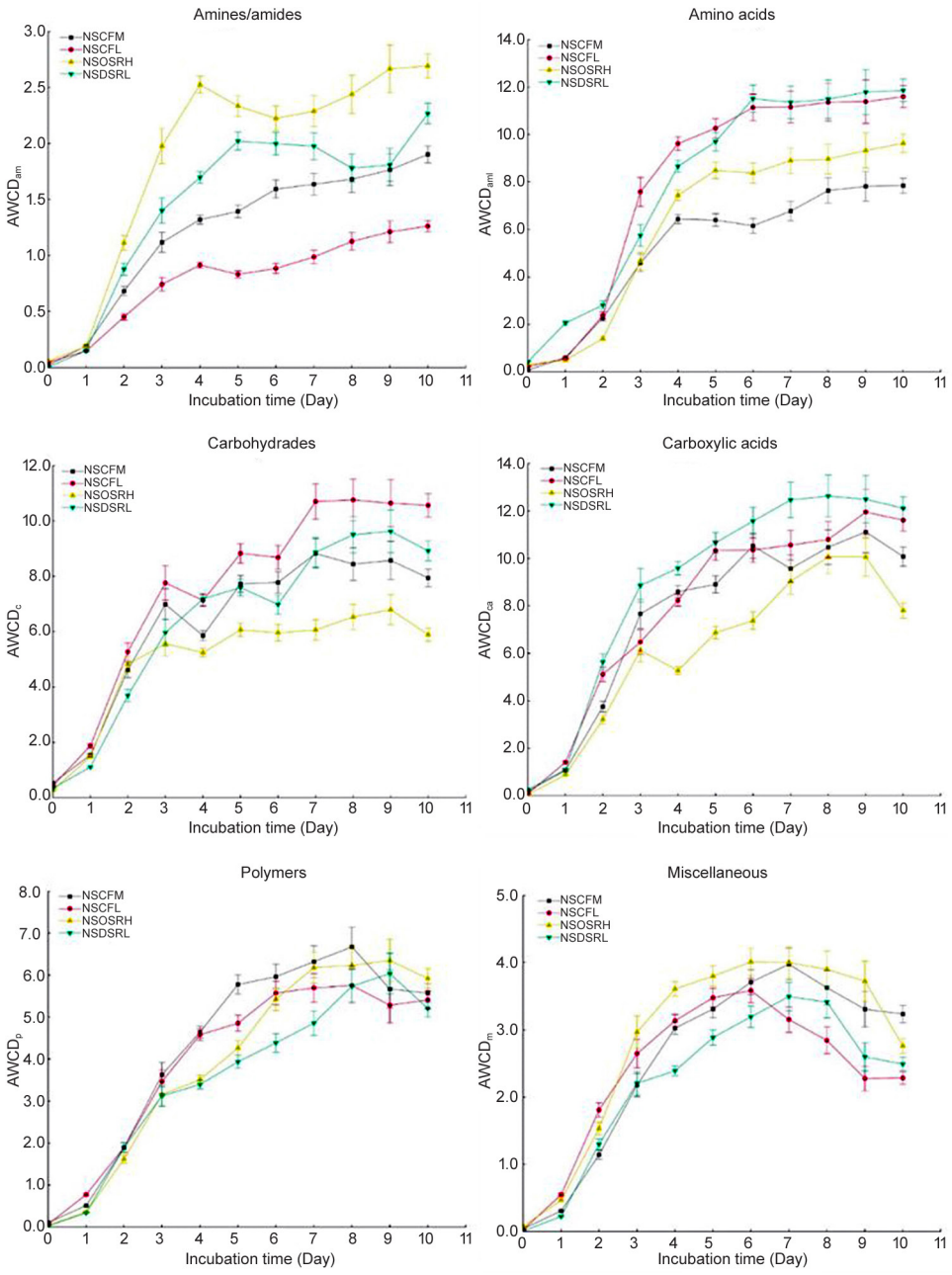

Fig. 2 The AWCD of six different classes of carbon substrates [class-specific substrates (i.e. amines/amides ($AWCD_{am}$), amino acids ($AWCD_{ami}$), carbohydrates ($AWCD_c$), carboxylic acids ($AWCD_{ca}$), polymers ($AWCD_p$), and miscellaneous ($AWCD_m$)] by rice rhizosphere microbial communities under high and low N and cultivation methods [(conventional flooding and DSR (aerobic)] during 10-day incubation time.
Values on the *y*-axis in each panel are unique to each class of carbon substrates. Treatment details are given under Materials and Methods.

tween 80, and tween 40. Enhanced degradation of amino sugars and polyols reflected the enhanced microbial adaptation to N-limited, anoxic soils (Anning *et al.* 2021). Tween utilisation is consistent with shifts in lipid handling under C: N imbalance reported for microbial communities (Dresser *et al.* 2022), although the EcoPlate assay itself only records community-level substrate oxidation. Enhanced amino acid catabolism may indicate greater use of amino acids as joint C and N sources under N deficiency, rather than directly evidencing specific N-scavenging pathways. Thus, the CFLN fostered resilient, oligotrophic microbial communities under the low-N conventional flooded condition.

The DSRHN favoured the utilisation of D, L-α-glycerol phosphate, D-malic acid, putrescine, glucose-1-phosphate, glycogen, α-cyclodextrin, L-threonine, phenylethylamine, α-keto butyric acid, and β-hydroxy-glycyl-L-glutamic acid. This metabolic signature suggested the high-energy substrate processing by the copiotrophic microbial community (Fierer *et al.* 2007). Polyamine and cyclic compound utilisation indicated osmotic regulation and redox modulation (Dunn *et al.* 2023). Efficient glycolytic routing of glucose derivatives reflects elevated metabolic throughput (Nikel *et al.* 2015). The DSRLN demonstrated broad utilisation of pyruvic acid methyl ester, itaconic acid, 2-hydroxy benzoic acid, D-glucosaminic acid, α-keto butyric acid, 4-hydroxy benzoic acid, D-cellobiose, phenylethylamine, D-mannitol, and D-xylose. The catabolism of benzoates, keto acids, and amino sugars suggested oxidative stress adaptation and flexible C acquisition (Parales and Harwood 2002). Under low-N, selection favoured the metabolic flexibility of the microbial communities that upregulate pathways to exploit non-preferred or complex substrates, thereby sustaining activity and nutrient acquisition. Thus, the DSRLN supported versatile, stress-resilient microbial assemblages.

Divergence of microbial metabolic fingerprints: PCA biplot (Fig. 4) showed the CSUP across treatments, with PC1 (68.5%) and PC2 (14.9%) together explaining 83.4% of the variance. PC1 primarily segregated the rhizosphere microbial communities by the N availability and the

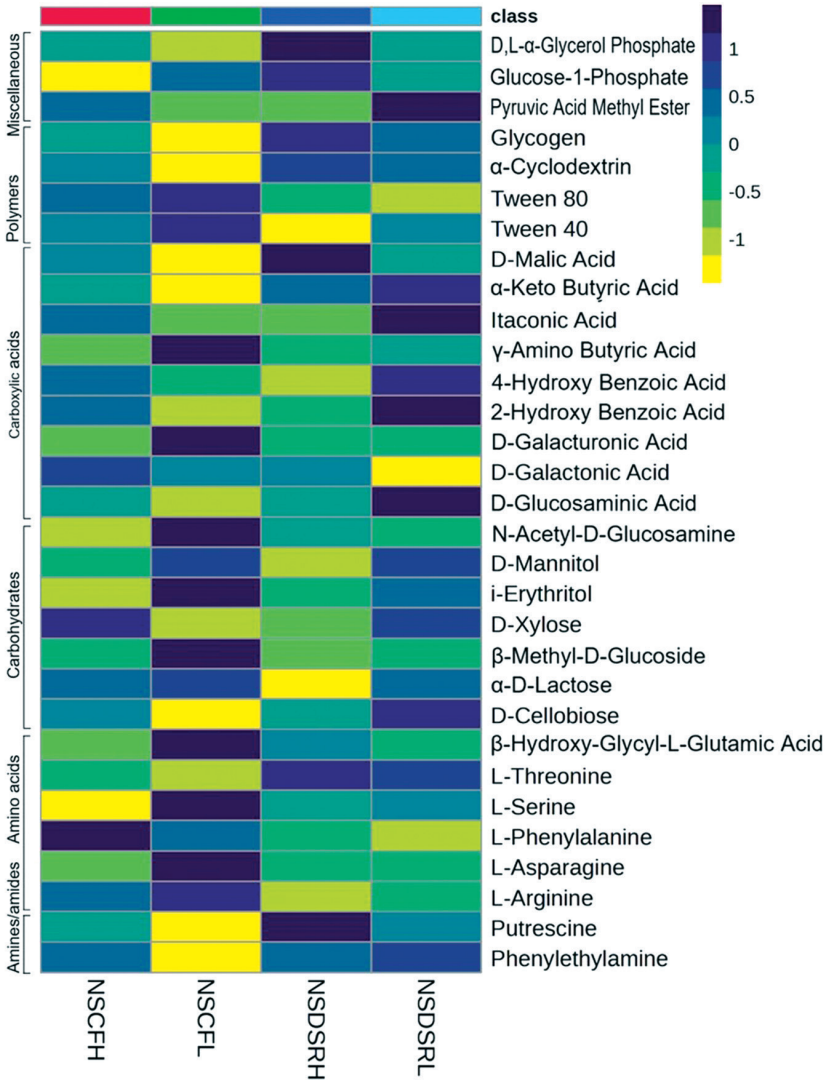

Fig. 3 The AWCD of 31 different carbon substrates by rice rhizosphere microbial communities.
Treatment details are given under Materials and Methods.

cultivation method, with CFLN (CF, low-N) clustering negatively, and DSR treatments (DSRHN, DSRLN) aligning positively, underscoring the decisive role of aerobic (oxygenation) condition in shaping microbial metabolic potential (Keiluweit *et al.* 2016). PC2 resolved finer metabolic shifts: CFLN's negative positioning reflected elevated amino acid catabolism typical of N-scarce, anoxic systems, whereas positive scores for DSRHN and DSRLN indicated enhanced catabolism of carboxylic acids, amines, and mixed substrates under aerobic, N-variable conditions. The substrate vectors reinforced these patterns, with amino acids and carbohydrates diverging along PC2, while carboxylic acids and amines loaded positively on PC1, reflecting oxidative, high-N catabolic versatility. Polymer aligned with CFHN and negative PC2 suggested fermentative degradation under flooded, N-rich conditions (Soong *et al.* 2018). Tight CFLN clustering indicated the metabolic potential of low-diversity, substrate specialist assemblages. In contrast, the DSRHN and DSRLN ellipses revealed elevated functional plasticity, probably driven by root exudates and redox heterogeneity. Carbohydrate utilisation was negatively correlated with the degradation of complex polymers, indicating a trade-off between reliance on readily available labile sugars and investment in extracellular enzyme production for depolymerisation. In contrast, carboxylic acid utilisation was negatively correlated with amino acid metabolism, reflecting a strong inverse relationship between oxidative organic acid catabolism and amino acid-driven deamination and fermentation. Collectively, these trends suggested that the N availability and aeration are significant ecological filters shaping microbial carbon source utilisation and metabolic activities, with cascading effects on organic matter turnover, N cycling, and resource optimisation in rice agroecosystems. The Biolog EcoPlate CLPP provided high-resolution fingerprints of potential substrate use under standardized conditions, complementing but not substituting pathway-resolved

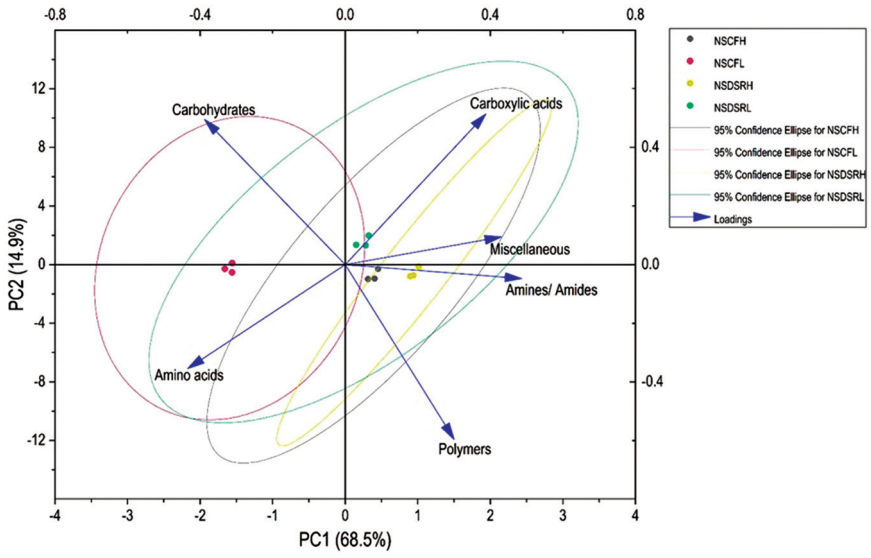

Fig. 4 Principal component analysis of carbon source utilisation patterns by the rice rhizosphere microbial communities.
Treatment details are given under Materials and Methods.

evidence from *in situ* flux and omics studies. Accordingly, our ecological inferences are framed as mechanistically informed, testable hypotheses that can be further examined using targeted metagenomic, transcriptomic, or metabolomic analyses.

The dynamics of rhizosphere microbial communities are informative regarding soil functions and productivity over time. The present study revealed that N availability and cultivation methods intricately modulated microbial carbon utilisation in rice rhizospheres. Under the DSR (aerobic) condition, low-N enhanced microbial metabolic flexibility, supporting amino acid and carbohydrate turnover. In contrast, high-N in the flooded conditions favoured aromatic and polymer degradation. The AWCD trends showed a shift from early metabolic lag to plateaued activity, reflecting substrate saturation and microbial adaptation. Functional diversity indices indicated treatment-specific community structure, with DSRLN exhibiting the most balanced assemblage. Carboxylic acids and amino acids emerged as preferential substrates, influenced by N constraints and energy demand, while the catabolism of amines was suppressed under anoxic conditions. PCA confirmed N and aeration as key ecological filters driving C metabolic divergence and niche-specific substrate utilisation. The distinct metabolic signatures reflected adaptive strategies of rhizosphere microbial communities. These findings offer insights into microbial metabolic activities and functional diversity, with implications for nutrient cycling, organic matter decomposition, and sustainable management of rice ecosystems and soil health.

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