



## RICE-STRAW INCORPORATION WITH A CELLULOLYTIC *BACILLUS* INOCULANT VERSUS STRAW BURNING: NITROGEN AND POTASSIUM SUPPLY TO THE FOLLOWING WHEAT CROP

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Received: 16-06-2026 Revised: 14-07-2026 Accepted: 04-10-2026

### ABSTRACT

Burning is the quickest way to clear rice straw before wheat, but it wastes nutrients and pollutes the air. Incorporating the straw keeps those nutrients on the field, yet its slow breakdown can lock up soil nitrogen (N) when young wheat needs it. We tested whether a cellulolytic *Bacillus* inoculant makes incorporation a better N and potassium (K) source than burning. A four-block field trial compared straw removal (T1), burning with the ash retained (T2), incorporation (T3) and incorporation plus *Bacillus* (T4), each with 6 t straw dry matter ha<sup>-1</sup>, 120 kg N ha<sup>-1</sup> and no mineral K. Litterbags, soil mineral N, exchangeable K and crop uptake were followed for one season. The inoculant raised the straw decay constant by 28% (half-life 67 vs 86 d) and turned early net N retention in the straw into net release. Incorporation lowered soil mineral N at sowing, but T4 held the most mineral N at stem elongation (25.6 mg kg<sup>-1</sup>) and the most exchangeable K at harvest (159 mg kg<sup>-1</sup>). Compared with burning, T4 took up 19.4 kg more N ha<sup>-1</sup> (+19.5%; Bonferroni-adjusted  $P < 0.001$ ) and 20.4 kg more K ha<sup>-1</sup> (+20.8%;  $P_{\text{adj}} = 0.028$ ), and grain yield rose by 8.5% ( $P_{\text{adj}} = 0.088$ ). A block-paired sensitivity test confirmed the N gain, strengthened the yield gain ( $P_{\text{adj}} = 0.013$ ) and weakened the K gain ( $P_{\text{adj}} = 0.21$ ). Inoculated incorporation is thus a credible replacement for burning, mainly through better N supply. Multi-season trials with labelled N are needed.

**Keywords:** Rice straw; Residue burning; Cellulolytic *Bacillus*; Litterbag decomposition; Nitrogen immobilization; Exchangeable potassium; Wheat nutrient uptake.

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DOI: <https://doi.org/10.37022/jis.v9i2.159>

### Produced and Published by

South Asian Academic Publications

### INTRODUCTION

Rice–wheat rotations leave a great deal of straw behind, and the gap between rice harvest and wheat sowing is short. Burning is therefore common; about 50 Mt of rice straw are burned every year in India alone [1]. Rice residues carry on average about 0.7% N and 1.75% K [2], and much of the straw C, N and S goes up in smoke [3]. In China, straw-burning emissions were linked to several thousand premature deaths in a single year [4]. In the soil, burning lowered available N by 12–14% in a rice–wheat trial while K changed only slightly [5], and it cut soil organic matter by about a quarter in wheat fields of northern Iraq [6]. Incorporation keeps these nutrients on the field, but it brings its own problem. Rice straw has a wide C:N

ratio, so the microbes that decompose it also take up mineral N. Wheat sown into fresh straw can then run short of N early in the season, and small yield penalties have been reported [7, 8]. Potassium is different: it leaves returned straw faster than any other major nutrient [9], and straw incorporation improved the K status of a rice–wheat soil over five years [10].

Decomposer inoculants may speed things up. In a two-year wheat trial, straw amended with a *Bacillus subtilis* inoculant raised crop productivity and the ecosystem C budget [11]. Among several microbial agents added to rice straw, *B. subtilis* was the most effective decomposer [12], and inoculation reshaped the lignocellulose-degrading community of paddy fields [13]. Still, few field studies have set inoculated incorporation directly against burning while tracing both N and K from the straw, through the soil, into the next wheat crop. We asked whether a cellulolytic *Bacillus* inoculant would (i) speed straw decay and nutrient release, (ii) shorten the early N lock-up and (iii) raise wheat N and K uptake compared with burning.

## 2. MATERIALS AND METHODS

### 2.1. Site, soil and experimental design

The field trial was run during the 2023–2024 wheat season at the Rice Research Station, Al-Mishkhab, Najaf Governorate, Iraq (31.85° N, 44.45° E, 18 m a.s.l.), on an irrigated loam soil that had carried transplanted rice in the preceding summer. Properties of the 0–20 cm layer are given in Table I (Table S1). The design was a randomized complete block with four treatments and four blocks. Each treatment appeared once in every block, giving 16 independent plots (Fig. 1b). Plots measured 5 m × 4 m (20 m<sup>2</sup>), and grain and straw were harvested from a net area of 12 m<sup>2</sup> in the plot centre.

Table 01: Initial properties of the soil (0–20 cm) and of the rice straw used in the trial.

| Property                                                                                   | Unit                               | Value                               |
|--------------------------------------------------------------------------------------------|------------------------------------|-------------------------------------|
| <b>Soil, 0–20 cm, before the residue treatments</b>                                        |                                    |                                     |
| Texture class                                                                              | —                                  | Loam (38% sand, 40% silt, 22% clay) |
| pH (1:2.5 soil:water)                                                                      | —                                  | 7.7                                 |
| EC (saturated-paste extract)                                                               | dS m <sup>-1</sup>                 | 1.2                                 |
| Organic C                                                                                  | g kg <sup>-1</sup>                 | 7.5                                 |
| Mineral N (NO <sub>3</sub> <sup>-</sup> -N + NH <sub>4</sub> <sup>+</sup> -N) <sup>a</sup> | mg kg <sup>-1</sup>                | 14.0 ± 1.2                          |
| Nitrate-N <sup>a</sup>                                                                     | mg kg <sup>-1</sup>                | 10.3 ± 0.9                          |
| Ammonium-N <sup>a</sup>                                                                    | mg kg <sup>-1</sup>                | 3.7 ± 0.4                           |
| Exchangeable K (1 M NH <sub>4</sub> OAc) <sup>a</sup>                                      | mg kg <sup>-1</sup>                | 143.3 ± 6.5                         |
| Bulk density                                                                               | g cm <sup>-3</sup>                 | 1.32                                |
| Cation exchange capacity                                                                   | cmol <sub>c</sub> kg <sup>-1</sup> | 24.6                                |
| Available P (Olsen)                                                                        | mg kg <sup>-1</sup>                | 11.8                                |
| <b>Rice straw (returned in T3 and T4)</b>                                                  |                                    |                                     |
| Dry matter load                                                                            | t ha <sup>-1</sup>                 | 6.0                                 |
| Carbon                                                                                     | g kg <sup>-1</sup> DM              | 400                                 |
| Nitrogen                                                                                   | g kg <sup>-1</sup> DM              | 6.5                                 |
| Potassium (elemental K)                                                                    | g kg <sup>-1</sup> DM              | 16.0                                |
| C:N ratio                                                                                  | —                                  | 61.5                                |
| C, N and K returned with the straw                                                         | kg ha <sup>-1</sup>                | 2400, 39 and 96                     |

<sup>a</sup> Mean ± SD of the 16 plots at -30 DAS (before the residue treatments).

### 2.2. Rice straw and residue treatments

The straw load was set to 6 t dry matter (DM) ha<sup>-1</sup> on every plot after the rice harvest. The straw contained 400 g C, 6.5 g N and 16 g K kg<sup>-1</sup> DM (elemental K), which gives a C:N ratio of 61.5 and pools of 39 kg N and 96 kg K ha<sup>-1</sup> (Table I). The four treatments were imposed 30 days before sowing (-30 days after sowing, DAS; Fig. 1a). In T1 the straw was removed from the plot. In T2 it was burned in place; the ash was left on the surface and then mixed in by shallow cultivation. In

T3 the straw was chopped to 5–8 cm and incorporated into the 0–15 cm layer with a rotary tiller. T4 was treated as T3 and also received the *Bacillus* inoculant. T1 received the same cultivation as the incorporated plots.

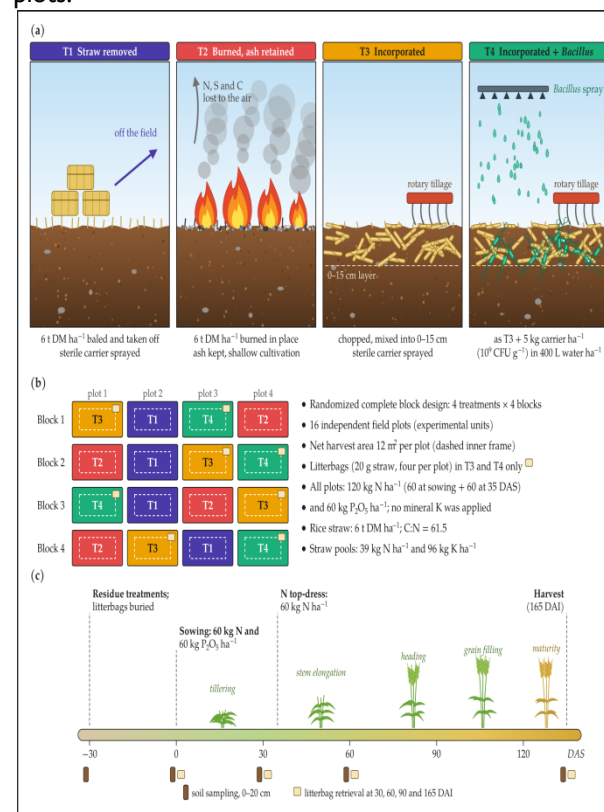


Figure 01: Experimental set-up. (a) The four rice-straw treatments, imposed 30 days before wheat sowing. (b)

Field plan of the randomized complete block design, showing the position of each plot within its block; small tags mark the plots that held litterbags. (c) Season timeline with residue treatment, fertilizer timing, soil sampling and litterbag retrieval. DAS, days after sowing; DAI, days after incorporation.

### 2.3. Inoculant

The inoculant was a cellulolytic *Bacillus subtilis* strain BS-12 (GenBank accession OP456789), isolated from rice-straw compost at the Department of Biology, College of Science, University of Baghdad formulated on a peat-based carrier. It was applied to T4 at 5 kg carrier ha<sup>-1</sup> with 10<sup>9</sup> viable cells (CFU) g<sup>-1</sup>, dispersed in 400 L water ha<sup>-1</sup> and sprayed onto the chopped straw just before incorporation, giving about 5 × 10<sup>12</sup> CFU ha<sup>-1</sup>. Plots T1–T3 received the same mass of sterilized carrier in the same volume of water, so that only the live cells differed between T3 and T4. No N was added with the inoculant. The viable count of the product was checked by plate counts on Luria–Bertani (LB) agar before use (1.2 × 10<sup>9</sup> CFU g<sup>-1</sup>).

### 2.4. Wheat management

Wheat (*Triticum aestivum* L., cv. Abu-Ghraib 3) was sown on 25 November 2023 at 140 kg ha<sup>-1</sup> in rows 17.5 cm apart. All plots received 120 kg N ha<sup>-1</sup> as urea (46% N), half at sowing and half at

35 DAS, and 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) at sowing. No mineral K was applied, so the crop depended on soil and residue K. Irrigation and weed and pest control followed local practice weeds were controlled by one hand weeding at 30 DAS followed by bromoxynil + MCPA at 0.75 L ha<sup>-1</sup> at 45 DAS; no significant pest or disease incidence was observed during the season. The crop was harvested at 135 DAS.

## 2.5. Litterbag decomposition

Straw decomposition was followed with the litterbag method [14]. Nylon bags (1 mm mesh, 20 × 15 cm) were filled with 20 g DM of the same chopped straw. The straw in the T4 bags was sprayed with the inoculant suspension at the field rate and the T3 straw with sterile carrier at the same rate. Four bags were buried at 0–15 cm in each T3 and T4 plot at incorporation, and one bag per plot was retrieved at 30, 60, 90 and 165 days after incorporation (DAI), that is, at 0, 30, 60 and 135 DAS. The residue was cleaned of soil and roots, oven-dried at 65 °C, weighed and analysed for N and K. Net nutrient disappearance was calculated as:

$$DX = 1 - (M_t \times X_t) / (M_0 \times X_0) \quad (1)$$

where M<sub>0</sub> and M<sub>t</sub> are the initial and remaining dry masses (g) and X<sub>0</sub> and X<sub>t</sub> the nutrient concentrations (g kg<sup>-1</sup>). A negative DN means that the residue gained N (net immobilization). Mass loss was described with a single first-order model and with an asymptotic first-order model [15, 16]:

$$M_t / M_0 = \exp(-kt) \quad (2)$$

$$M_t / M_0 = a + (1 - a) \exp(-kat) \quad (3)$$

where k and ka are decay constants (d<sup>-1</sup>), t is time (d) and a is the fraction of the mass that resisted decay during the study. The half-life was  $t_{1/2} = \ln 2/k$ . Both models were fitted to each plot by non-linear least squares.

## 2.6. Soil sampling and analysis

Soil (0–20 cm) was sampled in every plot at –30 DAS (before the residue treatments), 0 DAS (before basal N), 30 DAS (before the top-dress), 60 DAS (25 days after the top-dress) and 135 DAS (harvest); five cores were bulked per plot. Nitrate-N and ammonium-N were extracted with 2 M KCl and measured by steam distillation on a Kjeltex 8400 Auto Distillation Unit (FOSS, Denmark); mineral N is their sum. Exchangeable K was extracted with 1 M NH<sub>4</sub>OAc at pH 7 and read by flame photometry (Sherwood Model 420, UK). Concentrations are given per kg of oven-dry soil. Soil pH was measured in a 1:2.5 soil:water suspension, electrical conductivity (EC) in the saturated-paste extract and organic C by the Walkley–Black wet-oxidation method.

## 2.7. Harvest measurements and nutrient uptake

Plant height and spike density were recorded on 20 plants and 1 m<sup>2</sup> per plot before harvest. Grain and straw from the net area were weighed, grain moisture was measured with a Dickey-John GAC 2100 grain moisture tester, and a straw subsample was oven-dried

to constant weight. Grain yield was expressed at 12% moisture:

$$GY12 = Mg (1 - \theta) / (1 - 0.12) \times 10 / A \quad (4)$$

where Mg is the harvested grain mass (kg),  $\theta$  the measured moisture (fraction) and A the net area (12 m<sup>2</sup>); GY12 is in t ha<sup>-1</sup>. Thousand-grain weight was also expressed at 12% moisture. Grain and straw N were determined by the micro-Kjeldahl method and K by flame photometry after wet digestion with a H<sub>2</sub>SO<sub>4</sub>–H<sub>2</sub>O<sub>2</sub> mixture. Nutrient uptake (kg ha<sup>-1</sup>) was dry biomass (t ha<sup>-1</sup>) × concentration (g kg<sup>-1</sup>), and aboveground uptake was the sum of grain and straw; roots were not included. The harvest index was dry grain/(dry grain + dry straw), and the N and K harvest indices (NHI, KHI) were the grain share of aboveground uptake. We also report the nutrient requirement per tonne of grain (reciprocal internal use efficiency, RIUE, kg Mg<sup>-1</sup>) [17] and the partial factor productivity of fertilizer N (PFPN = GY12/120 kg N).

## 2.8. Statistical analysis

The 16 plots were the experimental units. Three primary outcomes were fixed before the analysis: grain yield, aboveground N uptake and aboveground K uptake. Each was analysed with the model  $y = \mu + \text{treatment} + \text{block} + \varepsilon$  (treatment and block df 3, error df 9). The main question was answered by one planned contrast, T4 minus T2. Its P value was Bonferroni-adjusted for the three outcomes ( $P_{\text{adj}} = \min[1, 3P]$ ), and family-wise 95% confidence intervals used  $t_{(1-0.05/6; 9)} = 2.933$ . Secondary crop traits, and the soil variables at each date, were analysed with the same model, and means were separated with Tukey's HSD ( $\alpha = 0.05$ ). Residual normality was checked with the Shapiro–Wilk test and homogeneity of variance with the Brown–Forsythe test. Because yield variance differed among treatments, the T4 – T2 contrast was also re-run as a paired t-test within blocks, which uses only the T2 and T4 plots (sensitivity analysis, Bonferroni-adjusted). Repeated soil samples and litterbags from the same plot were not counted as extra replicates. The treatment × date pattern of soil variables was tested with a linear mixed model with plot as a random effect. Decay parameters of T3 and T4 were compared with a paired t-test on block pairs (n = 4). Relations between variables were described with Pearson's r across the 16 plots. Analyses were run in Python with SciPy 1.18 and statsmodels 0.15. Values are means ± SD (n = 4).

3. RESULTS AND DISCUSSION

3.1. Straw decomposition and nutrient release

Table 02: Decomposition and nutrient release of incorporated rice straw in litterbags.

| Parameter                                 | T3 incorporated | T4 incorporated + <i>Bacillus</i> | P <sup>a</sup> |
|-------------------------------------------|-----------------|-----------------------------------|----------------|
| Dry-matter loss, 30 DAI (%)               | 25.9 ± 1.6      | 33.0 ± 0.8                        | 0.002          |
| Dry-matter loss, 60 DAI (%)               | 42.5 ± 2.6      | 48.2 ± 1.2                        | 0.004          |
| Dry-matter loss, 90 DAI (%)               | 52.7 ± 2.2      | 62.0 ± 1.6                        | 0.002          |
| Dry-matter loss, 165 DAI (%)              | 67.0 ± 2.0      | 73.7 ± 3.5                        | 0.039          |
| Straw N remaining, 30 DAI (% of initial)  | 102.6 ± 3.4     | 95.6 ± 1.1                        | 0.041          |
| Straw N remaining, 165 DAI (% of initial) | 63.3 ± 6.7      | 49.7 ± 5.1                        | 0.018          |
| Straw K released, 30 DAI (%)              | 72.5 ± 0.7      | 76.1 ± 1.1                        | 0.019          |
| Straw K released, 165 DAI (%)             | 96.3 ± 1.6      | 97.4 ± 0.9                        | 0.296          |
| Single first-order model, Eq. (2)         |                 |                                   |                |
| $k$ (d <sup>-1</sup> )                    | 0.0081 ± 0.0005 | 0.0104 ± 0.0004                   | 0.002          |
| $t_{1/2}$ (d)                             | 85.7 ± 5.3      | 66.9 ± 2.9                        | 0.004          |
| R <sup>2</sup>                            | 0.90 ± 0.03     | 0.86 ± 0.09                       | 0.351          |
| Asymptotic first-order model, Eq. (3)     |                 |                                   |                |
| $k_a$ (d <sup>-1</sup> )                  | 0.0142 ± 0.0011 | 0.0172 ± 0.0020                   | 0.065          |
| $a$ , resistant fraction                  | 0.26 ± 0.02     | 0.22 ± 0.06                       | 0.201          |
| R <sup>2</sup>                            | 0.995 ± 0.006   | 0.991 ± 0.006                     | 0.476          |

Means ± SD (n = 4 plots). DAI, days after incorporation; 30 DAI was the day of sowing. <sup>a</sup> Paired t-test on block pairs. The inoculant clearly sped up straw breakdown (Fig. 2a; Table 2). Inoculated straw lost 33% of its mass in the first 30 days, against 26% for straw without it (P = 0.002). The gap held until the last retrieval, when 74% and 67% of the mass had gone. The single first-order constant was 0.0104 d<sup>-1</sup> for T4 and 0.0081 d<sup>-1</sup> for T3, a

28% rise (P = 0.002), and the half-life fell from 86 to 67 days (P = 0.004). This model fitted only moderately (R<sup>2</sup> 0.86–0.90) because decay slowed after the first two months. The asymptotic model described the curves far better (R<sup>2</sup> ≥ 0.98) and suggested that about 22–26% of the mass resisted decay during the season (Table 2). The size of the effect is in line with recent work. A composite *Bacillus* inoculant raised straw degradation by 31.5% over 90 days [18], and a consortium of four *Bacillus* strains raised rice straw degradation by 37% and N release by 16% [19]. In paddy fields, a microbial decomposition agent combined with deep rotary tillage shortened the time to 50% decomposition to 81 days [16]. *Bacillus* cellulases hydrolyse and loosen lignocellulosic biomass [20], and inoculation with cellulolytic microbes also speeds the decay of other residues with a high C:N ratio [21].

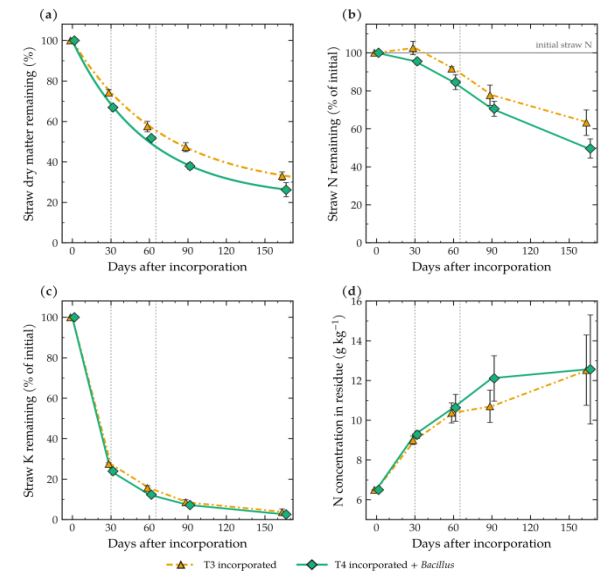


Figure 02: Decomposition and nutrient release of incorporated rice straw in litterbags. (a) Dry matter remaining, with asymptotic first-order fits (Eq. 3); (b) N remaining as a percentage of the initial straw N (values above 100% indicate net immobilization); (c) K remaining; (d) N concentration in the residue. Points are means ± SD (n = 4 plots); dotted lines mark wheat sowing (30 DAI) and the N top-dress (65 DAI). Nitrogen did not follow the mass (Fig. 2b). Straw without the inoculant gained N during the first 30 days (102.6% of its initial N, i.e. net immobilization), whereas inoculated straw had already lost 4.4% (P = 0.041). By harvest, T4 had released 50% of the straw N and T3 37% (P = 0.018), roughly 19.6 and 14.3 kg N ha<sup>-1</sup>. The N concentration of the residue roughly doubled in both treatments (Fig. 2d), because C was lost faster than N. Potassium, in contrast, left the straw quickly in both treatments (Fig. 2c). About 73–76% of the K had gone by sowing and more than 96% by harvest; the inoculant added only a little (76% vs 73% by sowing; P = 0.019). These patterns match the release order K > P > C > N reported for returned straw [9] and a two-year litterbag study in which 93.5% of rice straw K was released in the first month [22].

Maize straw lost 93–97% of its K in a similar test [23]. Most K in straw is soluble  $K^+$ , so it washes out without any microbial attack.

### 3.2. Soil Mineral Nitrogen

Mineral N was similar in all plots before the treatments (13.4–14.8  $mg\ kg^{-1}$ ;  $P = 0.34$ ; Table 3). Thirty days later, at sowing, both incorporation treatments held less mineral N than the plots without straw (Fig. 3a). The fall was largest in T3 (12.1  $mg\ kg^{-1}$ , against 16.3  $mg\ kg^{-1}$  in T1), and T4 was in between (13.2  $mg\ kg^{-1}$ ). Relative to the starting value of each plot, mineral N dropped by about 1.6–1.7  $mg\ kg^{-1}$  in T3 and T4 but rose by 2.1–2.5  $mg\ kg^{-1}$  in T1 and T2 ( $P < 0.001$ ; Fig. 3b). Both nitrate and ammonium contributed to the drop. This is the classic N lock-up under a wide-C:N residue. Microbes growing on fresh straw take mineral N from the soil, which can lower available N at tillering and cut panicle numbers in the next crop [24]. Straw also reduces N losses by holding N in microbial biomass, and much of that N is released again later [25].

Table 03: Soil (0–20 cm) mineral N and exchangeable K at each sampling date.

| Date                                             | T1 removed    | T2 burned       | T3 incorpor ated | T4 incorp. + <i>Bacillus</i> | P      |
|--------------------------------------------------|---------------|-----------------|------------------|------------------------------|--------|
| <b>Mineral N (<math>mg\ kg^{-1}</math>)</b>      |               |                 |                  |                              |        |
| –30 DAS                                          | 14.2 ± 0.8    | 13.4 ± 1.8      | 13.8 ± 0.8       | 14.8 ± 1.2                   | 0.34   |
| 0 DAS                                            | 16.3 ± 1.3 a  | 15.9 ± 2.0 ab   | 12.1 ± 1.8 b     | 13.2 ± 1.4 ab                | 0.02   |
| 30 DAS                                           | 14.5 ± 1.0    | 14.5 ± 1.2      | 12.8 ± 0.5       | 14.0 ± 0.7                   | 0.10   |
| 60 DAS                                           | 21.2 ± 1.8 b  | 20.9 ± 1.6 b    | 22.7 ± 0.6 ab    | 25.6 ± 0.8 a                 | 0.00   |
| 135 DAS                                          | 7.0 ± 1.2 b   | 6.3 ± 1.2 b     | 7.9 ± 1.6 ab     | 9.9 ± 1.2 a                  | 0.01   |
| <b>Exchangeable K (<math>mg\ kg^{-1}</math>)</b> |               |                 |                  |                              |        |
| –30 DAS                                          | 143.7 ± 5.9   | 147.0 ± 7.0     | 135.6 ± 1.6      | 147.1 ± 3.1                  | 0.05   |
| 0 DAS                                            | 143.0 ± 2.7 c | 176.3 ± 10.7 ab | 171.3 ± 5.7 b    | 183.8 ± 6.1 a                | < 0.00 |
| 30 DAS                                           | 131.1 ± 3.4 c | 165.1 ± 6.4 b   | 167.7 ± 5.1 ab   | 176.8 ± 4.8 a                | < 0.00 |
| 60 DAS                                           | 123.1 ± 8.0 b | 154.6 ± 4.8 a   | 154.5 ± 5.8 a    | 164.2 ± 5.6 a                | < 0.00 |
| 135 DAS                                          | 108.6 ± 8.9 c | 141.0 ± 7.4 b   | 141.3 ± 4.1 b    | 159.2 ± 6.4 a                | < 0.00 |

Means ± SD ( $n = 4$ ). Within a row, means sharing a letter do not differ (Tukey's HSD,  $\alpha = 0.05$ ); letters are

omitted when the treatment effect was not significant. DAS, days after sowing.

The picture changed after the top-dress. At 60 DAS, T4 had 25.6  $mg\ kg^{-1}$  of mineral N, about 4.3  $mg\ kg^{-1}$  more than T1 and 4.6  $mg\ kg^{-1}$  more than T2 ( $P = 0.006$ ), while T3 was intermediate (22.7  $mg\ kg^{-1}$ ). The treatment × date interaction was strong ( $\chi^2 = 108.6$ ,  $df = 9$ ,  $P < 0.001$ ). T4 also left the most mineral N at harvest (9.9 vs 6.3  $mg\ kg^{-1}$  in T2), and its area under the mineral-N curve from sowing to harvest was 16% larger than that of the burned plots ( $P = 0.043$ ). The litterbags help explain this, since inoculated straw began to release N before sowing and kept releasing it faster (Fig. 2b). Faster residue turnover may also have stimulated the mineralization of native soil organic N. Straw inputs can prime the decomposition of soil organic matter, and the size of this priming depends on N availability and on the microbes that assimilate straw C [26]. The burned plots, by contrast, behaved almost like the plots without straw. Long-term burned-straw return lowered the mineralizable N ratio of a paddy soil compared with straw return [27], and burning reduced available N, urease activity and microbial biomass C under wheat [28].

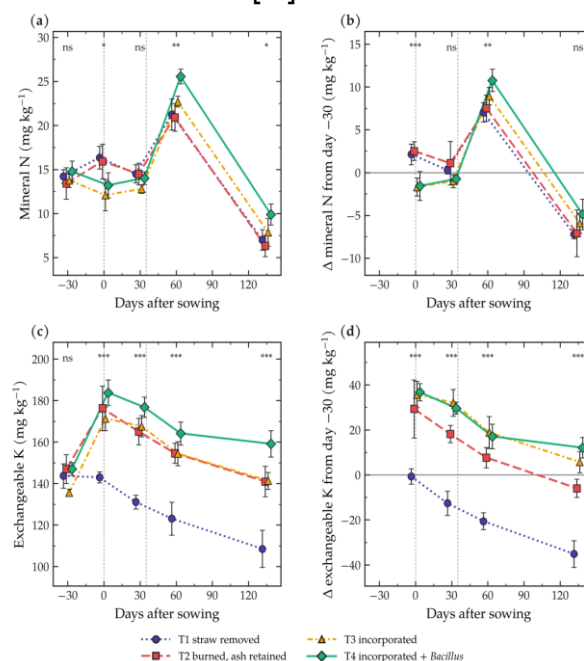


Figure 03: Soil (0–20 cm) mineral N (a) and exchangeable K (c) during the season, and their change from the pre-treatment value of the same plot (b, d). Means ± SD ( $n = 4$ ). Symbols above each date give the treatment effect in the RCBD ANOVA at that date (ns,  $P \geq 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

Dotted lines mark sowing with basal N (0 DAS) and the N top-dress (35 DAS).

### 3.3. Exchangeable potassium

Exchangeable K responded early and strongly (Fig. 3c, d). Before the treatments it ranged from 136 to 147  $mg\ kg^{-1}$  ( $P = 0.051$ ). By sowing it had risen by 29  $mg\ kg^{-1}$  in the burned plots and by 36–37  $mg\ kg^{-1}$  in the incorporated plots, but not where the straw had been removed (–0.7  $mg\ kg^{-1}$ ). The ash released its K at once,

and the straw released most of its K within the same 30 days, as the litterbags showed. Exchangeable K then declined at much the same rate in every treatment as the wheat took up K (treatment  $\times$  date,  $P = 0.056$ ). The gains did not last equally, though. At harvest the burned plots were  $6 \text{ mg kg}^{-1}$  below their starting level, while T4 was still  $12 \text{ mg kg}^{-1}$  above it ( $P < 0.001$ ). T4 ended with  $159 \text{ mg kg}^{-1}$ , against  $141 \text{ mg kg}^{-1}$  in T2 and T3 and  $109 \text{ mg kg}^{-1}$  in T1 (Table 3). Part of the T3–T4 gap at harvest reflects the lower starting values of the T3 plots; measured as change from the start, the two did not differ (Fig. 3d).

Ash K is soluble and lies at the surface, so part of it can be lost by runoff or leaching or move into less available pools. Burning raised available K at once in a Thai paddy, but the value had returned to its initial level a year later [29]. In an eight-year trial, rice residue incorporation kept more K in water-soluble and exchangeable forms than residue removal, whereas long-term burning reduced soil organic C and microbial biomass [30]. Straw return also improves the K supplying capacity of soils, with a large share of the added K entering the exchangeable pool [31]. Large soluble K inputs are not risk-free, however, since they can raise K losses by runoff and leaching [32].

### 3.4. Grain yield and yield components

Straw management affected grain yield ( $P = 0.022$ ; Fig. 4a; Table 4). T4 gave the highest yield,  $4.66 \text{ t ha}^{-1}$ , and was also the most uniform across blocks (SD  $0.04 \text{ t ha}^{-1}$ ). Burning gave  $4.29 \text{ t ha}^{-1}$ , incorporation alone  $4.24 \text{ t ha}^{-1}$  and removal  $4.13 \text{ t ha}^{-1}$ . The planned T4 – T2 contrast was  $0.36 \text{ t ha}^{-1}$  (+8.5%). Its raw  $P$  value was 0.029, but after the Bonferroni adjustment it was 0.088, and the family-wise 95% interval ran from  $-0.05$  to  $0.78 \text{ t ha}^{-1}$  (Table 5; Fig. 5d). The residuals were normal, but yield variance differed among treatments (Brown–Forsythe  $P < 0.001$ ); T3 varied widely and T4 hardly at all. A paired comparison of T4 and T2 within blocks, which does not borrow the T3 variance, gave the same difference with  $P_{\text{adj}} = 0.013$  (95% CI  $0.14$ – $0.59 \text{ t ha}^{-1}$ ). So the data point to a real yield gain, but its significance depends on the error model. T4 did out-yield T1, by  $0.53 \text{ t ha}^{-1}$  (Tukey  $P = 0.019$ ). Its higher yield came from more spikes ( $320$  vs  $295 \text{ m}^{-2}$  in T2) and heavier grains ( $41.6$  vs  $39.3 \text{ g}$ ), with no change in the harvest index ( $0.39$ – $0.40$ ). Straw dry matter followed the same order as grain (Fig. 4d), and the inoculated plots were also the tallest.

Table 04: Growth, yield components and yield of wheat after the four rice-straw treatments.

| Trait                                            | T1 removed                 | T2 burned                  | T3 incorporated             | T4 incorp. + <i>Bacillus</i> | P     | CV (%) |
|--------------------------------------------------|----------------------------|----------------------------|-----------------------------|------------------------------|-------|--------|
| Plant height (cm)                                | $78.5 \pm 2.6 \text{ c}$   | $82.2 \pm 2.4 \text{ bc}$  | $84.3 \pm 2.9 \text{ ab}$   | $87.8 \pm 2.0 \text{ a}$     | 0.002 | 2.8    |
| Spikes ( $\text{m}^{-2}$ )                       | $285.8 \pm 14.8 \text{ b}$ | $295.3 \pm 7.0 \text{ ab}$ | $311.0 \pm 15.8 \text{ ab}$ | $319.5 \pm 4.8 \text{ a}$    | 0.013 | 4.0    |
| Thousand-grain weight (g)                        | $39.8 \pm 1.4 \text{ ab}$  | $39.3 \pm 1.2 \text{ b}$   | $40.4 \pm 1.6 \text{ ab}$   | $41.6 \pm 0.7 \text{ a}$     | 0.035 | 2.4    |
| Grain yield, 12% moisture ( $\text{t ha}^{-1}$ ) | $4.13 \pm 0.15 \text{ b}$  | $4.29 \pm 0.13 \text{ ab}$ | $4.24 \pm 0.36 \text{ ab}$  | $4.66 \pm 0.04 \text{ a}$    | 0.022 | 4.6    |
| Straw dry matter ( $\text{t ha}^{-1}$ )          | $5.49 \pm 0.25 \text{ b}$  | $5.56 \pm 0.22 \text{ b}$  | $5.86 \pm 0.08 \text{ ab}$  | $6.17 \pm 0.33 \text{ a}$    | 0.010 | 4.1    |
| Aboveground dry matter ( $\text{t ha}^{-1}$ )    | $9.12 \pm 0.34 \text{ b}$  | $9.34 \pm 0.33 \text{ b}$  | $9.59 \pm 0.39 \text{ ab}$  | $10.27 \pm 0.33 \text{ a}$   | 0.005 | 3.5    |
| Harvest index (dry matter)                       | $0.398 \pm 0.009$          | $0.405 \pm 0.004$          | $0.389 \pm 0.017$           | $0.400 \pm 0.013$            | 0.371 | 3.1    |

Means  $\pm$  SD ( $n = 4$ ). Within a row, means sharing a letter do not differ (Tukey's HSD,  $\alpha = 0.05$ ).  $P$  is the treatment effect in the RCBD ANOVA (df 3, 9); CV is the residual coefficient of variation.

Table 05: Analysis of variance and the planned contrast (T4 – T2) for the three primary outcomes.

| Outcome                            | $F_{\text{treatment}} (P)$ | MSE    | CV (%) | T4 – T2 (95% CI) <sup>a</sup>   | Change (%) | $P_{\text{raw}}$ | $P_{\text{adj}}^{\text{b}}$ | $P_{\text{adj}}^{\text{paired}^{\text{c}}}$ |
|------------------------------------|----------------------------|--------|--------|---------------------------------|------------|------------------|-----------------------------|---------------------------------------------|
| Grain yield ( $\text{t ha}^{-1}$ ) | 5.30 (0.022)               | 0.0399 | 4.6    | $0.36 (-0.05 \text{ to } 0.78)$ | +8.5       | 0.029            | 0.088                       | 0.013                                       |
| N uptake ( $\text{kg ha}^{-1}$ )   | 18.88 (< 0.001)            | 20.47  | 4.3    | $19.4 (10.0 \text{ to } 28.7)$  | +19.5      | < 0.001          | < 0.001                     | 0.001                                       |
| K uptake ( $\text{kg ha}^{-1}$ )   | 6.10 (0.015)               | 76.66  | 8.2    | $20.4 (2.3 \text{ to } 38.6)$   | +20.8      | 0.009            | 0.028                       | 0.213                                       |

RCBD ANOVA with treatment df = 3, block df = 3 and error df = 9 ( $n = 16$  plots); block effects were not significant ( $P = 0.341$ ,  $0.369$  and  $0.926$ ). <sup>a</sup> Family-wise 95% confidence interval using  $t_{(1-0.05/6; 9)} = 2.933$ . <sup>b</sup> Bonferroni adjustment for three outcomes,  $P_{\text{adj}} = \min(1, 3P_{\text{raw}})$ . <sup>c</sup> Sensitivity analysis: paired  $t$ -test of T4 against T2 within blocks (df = 3), Bonferroni-adjusted. Change is relative to the T2 mean.

Incorporation without the inoculant did not raise yield over burning, and its yield was the most variable (SD  $0.36 \text{ t ha}^{-1}$ ); two of its four plots gave less than  $4.0 \text{ t ha}^{-1}$ . A wide-C:N residue that decays slowly gives an uneven N supply,

which is a likely source of this spread. Straw incorporation alone did not raise rice or wheat yields in a long-term trial in north-western India, although it did when combined with fertilizer N [33]. Residue retention raised wheat yield by 9.8–11.3% over burning and removal [34], and the yield loss after burning could not be offset by an extra 50 kg N ha<sup>-1</sup> [35]. Decomposer-assisted incorporation also raised wheat yield, straw yield and nutrient uptake compared with other straw management options [36]. Our 8.5% gain over burning sits within this range, although its statistical support depends on the error model.

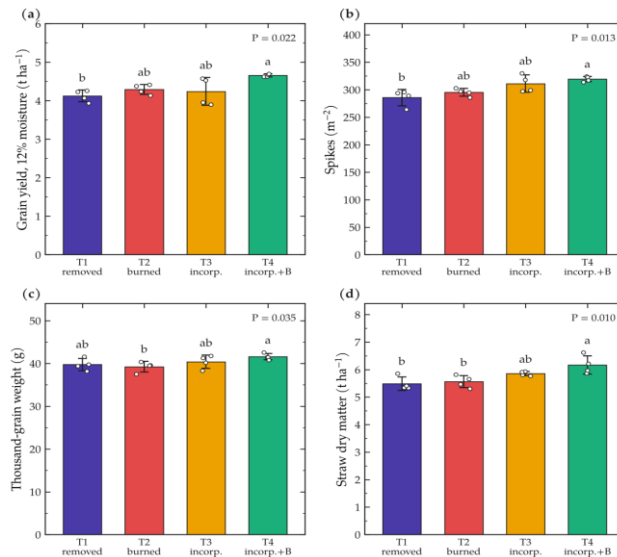


Figure 04: Grain yield at 12% moisture (a), spike density (b), thousand-grain weight (c) and straw dry matter (d) of wheat after the four straw treatments. Bars are means  $\pm$  SD ( $n = 4$ ) and open circles are individual plots. P values are for the treatment effect in the RCBD ANOVA; bars sharing a letter do not differ (Tukey's HSD,  $\alpha = 0.05$ ).

### 3.5. Nitrogen uptake

Nitrogen uptake gave the clearest response (Fig. 5a; Table 6). T4 took up 118.4 kg N ha<sup>-1</sup>, which was 19.4 kg ha<sup>-1</sup> (+19.5%) more than T2 ( $P_{adj} < 0.001$ ; 95% CI 10.0–28.7 kg ha<sup>-1</sup>). It was also 14.0 kg ha<sup>-1</sup> more than T3 (Tukey  $P = 0.008$ ), so the inoculant added to the effect of incorporation itself. T1, T2 and T3 did not differ (96.3–104.4 kg ha<sup>-1</sup>). The extra N in T4 came from both a higher grain N concentration (21.6 vs 20.1 g kg<sup>-1</sup> in T2) and more biomass, and it was shared between grain and straw in the usual way (NHI 0.73–0.77;  $P = 0.13$ ). Grain yield was closely tied to N uptake across the 16 plots ( $r = 0.88$ ,  $P < 0.001$ ; Fig. 5c), and N uptake in turn followed the mineral N at 60 DAS ( $r = 0.75$ ,  $P < 0.001$ ). The N supply around stem elongation, when wheat takes up N fastest, therefore seems to be the main link between straw management and yield in this trial. T4 used 25.4 kg N per tonne of grain against 23.1 kg in T2, and its partial factor productivity of fertilizer N rose from 35.8 to 38.8 kg grain kg<sup>-1</sup> N (Table 6).

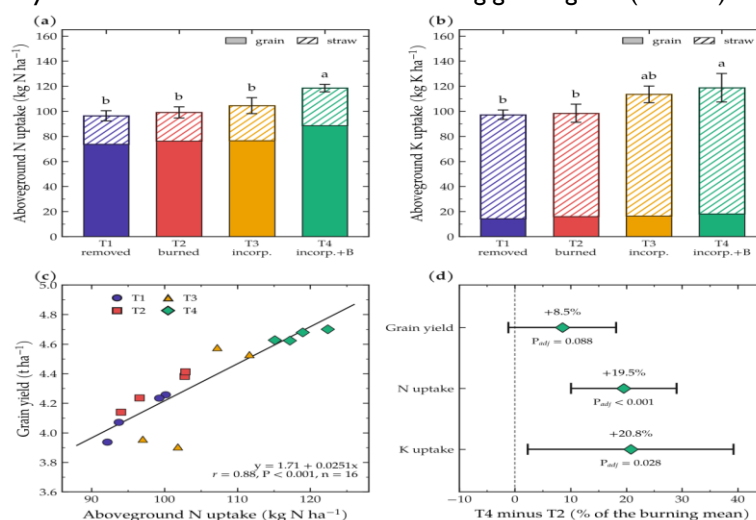


Figure 05: Aboveground N (a) and K (b) uptake of wheat divided into grain (solid) and straw (hatched); letters refer to total uptake (Tukey's HSD,  $\alpha = 0.05$ ). (c) Grain yield against N uptake for the 16 plots, with the fitted linear regression.

(d) The planned contrast T4 – T2 for the three primary outcomes, as a percentage of the T2 mean, with Bonferroni family-wise 95% confidence intervals.

It is tempting to read the extra uptake as straw N, but the numbers do not support that. Inoculated straw released only about 5.3 kg N ha<sup>-1</sup> more than plain straw by harvest, well below the 14.0 kg ha<sup>-1</sup> difference in uptake between T4 and T3. Labelled-N studies point the same way. Mulched straw supplied less than 1% of the N taken up by rice, and soil N was the main source [37]. Incorporated straw contributed little N to rice directly, yet it controlled crop N nutrition through microbial feedback and priming [38]. Better root growth and less immobilization of fertilizer N may also have helped T4. In a ten-year field and simulation study, rice straw incorporation raised wheat N uptake and N recovery and reduced N leaching and NH<sub>3</sub> losses [39]. We did not use a tracer, so the sources of the extra N remain open.

Table 06: Nitrogen and potassium concentration, uptake and use indices of wheat.

| Variable                                        | T1 removed     | T2 burned      | T3 incorporated | T4 incorp. + <i>Bacillus</i> | P       |
|-------------------------------------------------|----------------|----------------|-----------------|------------------------------|---------|
| <b>Concentration (g kg<sup>-1</sup> DM)</b>     |                |                |                 |                              |         |
| Grain N                                         | 20.28 ± 0.30 b | 20.12 ± 0.40 b | 20.53 ± 0.63 ab | 21.59 ± 0.36 a               | 0.009   |
| Straw N                                         | 4.14 ± 0.09    | 4.13 ± 0.42    | 4.76 ± 0.38     | 4.86 ± 0.42                  | 0.046   |
| Grain K                                         | 3.92 ± 0.05 c  | 4.22 ± 0.13 b  | 4.40 ± 0.17 a   | 4.43 ± 0.12 a                | < 0.001 |
| Straw K                                         | 15.14 ± 0.79   | 14.80 ± 0.80   | 16.56 ± 0.96    | 16.27 ± 1.03                 | 0.057   |
| <b>Uptake (kg ha<sup>-1</sup>)</b>              |                |                |                 |                              |         |
| Grain N                                         | 73.6 ± 3.2 b   | 76.0 ± 2.0 b   | 76.6 ± 6.0 b    | 88.5 ± 1.9 a                 | 0.001   |
| Straw N                                         | 22.7 ± 1.4 b   | 23.0 ± 3.2 b   | 27.9 ± 2.2 ab   | 29.9 ± 2.2 a                 | 0.007   |
| Aboveground N                                   | 96.3 ± 4.0 b   | 99.1 ± 4.5 b   | 104.4 ± 6.4 b   | 118.4 ± 3.1 a                | < 0.001 |
| Grain K                                         | 14.2 ± 0.4 c   | 15.9 ± 0.7 bc  | 16.4 ± 1.6 ab   | 18.1 ± 0.5 a                 | 0.001   |
| Straw K                                         | 83.0 ± 4.0     | 82.4 ± 6.6     | 97.0 ± 5.9      | 100.6 ± 11.7                 | 0.025   |
| Aboveground K                                   | 97.2 ± 3.8 b   | 98.3 ± 7.2 b   | 113.4 ± 6.7 ab  | 118.7 ± 11.4 a               | 0.015   |
| <b>Indices</b>                                  |                |                |                 |                              |         |
| N harvest index                                 | 0.764 ± 0.011  | 0.768 ± 0.024  | 0.733 ± 0.022   | 0.747 ± 0.014                | 0.126   |
| K harvest index                                 | 0.147 ± 0.009  | 0.162 ± 0.005  | 0.145 ± 0.011   | 0.154 ± 0.016                | 0.157   |
| RIUE <sub>N</sub> (kg N Mg <sup>-1</sup> grain) | 23.3 ± 0.2 bc  | 23.1 ± 0.4 c   | 24.7 ± 1.1 ab   | 25.4 ± 0.5 a                 | 0.003   |
| RIUE <sub>K</sub> (kg K Mg <sup>-1</sup> grain) | 23.6 ± 1.5     | 22.9 ± 1.4     | 26.8 ± 2.3      | 25.5 ± 2.5                   | 0.071   |
| PPF <sub>N</sub> (kg grain kg <sup>-1</sup> N)  | 34.4 ± 1.3 b   | 35.8 ± 1.1 ab  | 35.4 ± 3.0 ab   | 38.8 ± 0.3 a                 | 0.022   |

Means ± SD (n = 4). Within a row, means sharing a letter do not differ (Tukey's HSD,  $\alpha = 0.05$ ); letters are omitted when the treatment effect was not significant or when Tukey's test found no pairwise difference. RIUE, reciprocal internal use efficiency (aboveground uptake per tonne of grain at 12% moisture); PPF<sub>N</sub>, partial factor productivity of fertilizer N.

### 3.6. Potassium uptake and the K balance

Potassium uptake followed a similar order (Fig. 5b). T4 took up 118.7 kg K ha<sup>-1</sup>, 20.4 kg ha<sup>-1</sup> (+20.8%) more than T2 ( $P_{\text{adj}} = 0.028$ ; 95% CI 2.3–38.6 kg ha<sup>-1</sup>). This contrast was less stable than the N contrast. In the block-paired analysis it was not significant ( $P_{\text{adj}} = 0.21$ ), because block 1 alone gave a 41 kg ha<sup>-1</sup> difference against 7–21 kg ha<sup>-1</sup> in the other blocks. Incorporation without the inoculant gave 113.4 kg K ha<sup>-1</sup>, which did not differ from T4 (Tukey  $P = 0.82$ ). For K, then, most of the benefit came from returning the straw rather than from the inoculant. About 85% of the K stayed in the straw (KHI 0.14–0.16), as is usual for wheat. Grain K concentration was lowest where straw had been removed (3.92 g kg<sup>-1</sup>) and highest in T3 and T4 (4.40–4.43 g kg<sup>-1</sup>). Crop K uptake was related to exchangeable K at harvest ( $r = 0.66$ ,  $P = 0.006$ ). The crop needed 22.9–26.8 kg K per tonne of grain, close to the 20.3–25.1 kg reported for wheat in a rice–wheat system [10].

With no mineral K applied, the rice straw was the only external K input. If the wheat straw is also taken off the field, the apparent K balance of the season was –97 kg ha<sup>-1</sup> in T1, but only –17 and –23 kg ha<sup>-1</sup> in T3 and T4. If the wheat straw stays on the field, the balance becomes positive in the incorporated plots (+78 to +80 kg ha<sup>-1</sup>). The balance for T2 depends on how much ash K stayed on the plots, which we did not measure. Straw return alone does not always stop soil K depletion [40], but straw K can replace about half of the K fertilizer in some systems [41], and residue retention reduced K mining in a rice–wheat system [17]. Here, returning the rice straw covered most of the K removed by the next wheat crop.

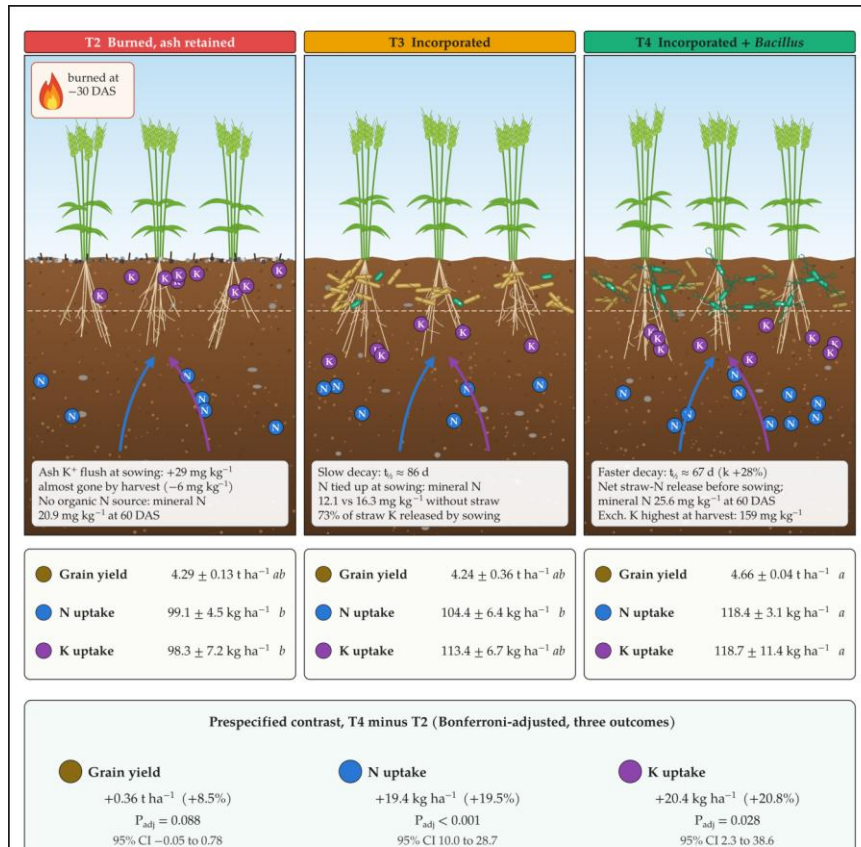


Figure 06: Pictorial summary of how the three residue systems supplied N and K to the following wheat. Values are from this study (means ± SD, n = 4; letters from Tukey's HSD); arrows show crop uptake of N (blue) and K (purple). The lower panel gives the planned T4 – T2 contrast with Bonferroni-adjusted P values.

### 3.7. Practical meaning and limitations

Taken together, the results suggest a simple sequence (Fig. 6). Burning keeps the ash K but loses the straw N and C, so the wheat relies on fertilizer N and native soil N. Plain incorporation keeps both nutrients, but its slow decay locks up N early and makes the response uneven. Adding the *Bacillus* inoculant shortens the lock-up, brings N release closer to the period of fast crop uptake and keeps exchangeable K higher until harvest. Two practical points follow. First, a larger share of basal N may still help under plain incorporation; it removed early N stress in super-seeder wheat sown into rice residue [8], and co-applying N with a decomposer inoculant improved straw decay [42]. Second, when burning is replaced by incorporation, farm K budgets should count the straw K.

The study has clear limits. It covers one season, one soil and one inoculant dose, with four blocks, and the planned and paired analyses disagreed for yield and K. With the pooled error, the trial had only about 56% power to detect a true 10% yield gain at the adjusted level, and about 14% for a 5% gain. We did not measure the survival of the inoculated strain, soil enzymes or microbial biomass, the K retained in the ash, N losses during burning or soil bulk density, so soil pools could not be converted to kg ha<sup>-1</sup>. Litterbags show what disappears from the residue, not what the crop recovers. Firm advice will need multi-season trials

with <sup>15</sup>N-labelled straw, plus ash sampling and a simple cost analysis.

### 4. CONCLUSION

This one-season field study compared four ways of handling 6 t ha<sup>-1</sup> of rice straw before wheat. A cellulolytic *Bacillus* inoculant made incorporated straw decay faster, with a 28% higher decay constant and a half-life of 67 instead of 86 days. It also turned the early net N retention in the straw into net release. Incorporation lowered soil mineral N at sowing, but the inoculated plots had the most mineral N at stem elongation and the most exchangeable K at harvest. Compared with burning, inoculated incorporation raised wheat N uptake by 19.5%, and this gain held under both the planned and the block-paired analysis. Potassium uptake (+20.8%) and grain yield (+8.5%) also rose, but each was significant under only one of the two analyses, so both should be treated as likely rather than proven. Plain incorporation kept the straw K but gave an uneven N supply and no clear yield benefit. Inoculated incorporation is therefore a sound alternative to burning in rice–wheat systems, mainly because it delivers more N and K to the next crop. Longer trials with <sup>15</sup>N tracing, some of them on farmers' fields, should now test the yield effect and the economics.

## 5. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## 6. DATA AVAILABILITY

The plot-level data and the R scripts used for the statistical analysis are available from the corresponding author on reasonable request.

## 7. ACKNOWLEDGEMENTS

This work was supported by the Iraqi Ministry of Higher Education and Scientific Research (grant no. 2023/AGR/112). The authors thank the staff of the Rice Research Station, Al-Mishkhab, for field management and Mr. Ali J. Kadhim for laboratory assistance.

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