

Effect of false-seedbed technique on weed management and soil quality in rice fields

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ABSTRACT

The increasing prevalence of herbicide resistance and the global shift toward reduced agrochemical usage necessitate innovative, sustainable weed management strategies. The false-seedbed technique is an eco-friendly approach, has gained prominence in organic agriculture and herbicide-resistant weed management. However, impacts of the false-seedbed technique on weed control efficacy and soil health remain underexplored. This study evaluated the effects of varying false-seedbed parameters (e.g., 0, 20, 30, 40 d induction; and tillage once or twice) on weed suppression, soil physicochemical properties, and microbial diversity in rice (*Oryza sativa* L.) fields. Key findings revealed that a 40 d induction achieved approximately 75% weed control efficacy, compared to the no-induction treatment, which was lower than conventional herbicide treatments. Prolonged induction periods significantly enhanced the accumulation of total organic C (TOC) and total N (TN), but had no effect on total P (TP) or total K (TK). Notably, herbicide application reduced soil microbial richness and diversity, whereas the false-seedbed technique preserved microbial community structure across all treatments. Tillage once or twice showed nonsignificant influence on weed control or soil quality metrics. These results demonstrate that the false-seedbed technique is a viable, eco-friendly alternative to herbicides, balancing effective weed management with the preservation of soil health. Its integration into rice farming systems could contribute to sustainable agriculture by minimizing agrochemical dependency and maintaining soil biodiversity. To validate the trends observed in this case study, longer-time and field-to-field replication is required.

Key words: False-seedbed technique, soil microbial community, soil physicochemical properties, weed control.

INTRODUCTION

Crop productivity faces significant threats from pests, particularly weeds, which compete for resources and reduce yields (Oerke, 2005). Rice (*Oryza sativa* L.), the staple food for over half of the global population, is highly vulnerable to weed infestations in agroecosystems. Globally, weeds are estimated to cause approximately 10% yield loss in rice production (Oerke and Dehne, 2004). To mitigate these challenges, diverse weed control strategies have been employed, ranging from chemical herbicides to agronomic practices (e.g., tillage, competitive cultivars), physical methods (e.g., mechanical weeders), and water management. Among these, herbicides remain the most widely used solution due to their rapid efficacy. The advantages of herbicides are still worth noting, e.g., high efficacy, specificity, relatively low cost, and reduced labor requirements. However, overreliance on herbicides has led to unintended ecological and agronomic consequences, including environmental contamination, human health risks, and the evolution of herbicide-resistant weed populations (Juliano et al., 2010). Alarming, cross-resistance and multiple-resistance mechanisms, where weeds developing tolerance to multiple herbicide modes of action, have become increasingly prevalent (Iwakami et

al., 2015; Riar et al., 2017; Fang et al., 2019). These challenges underscore the urgent need for integrated weed management (IWM) strategies that reduce chemical dependency while maintaining productivity and ecological sustainability.

The false-seedbed technique, inducing weed germination through optimized experimental conditions and then controlling weeds through tillage to reduce the seedbank (Travlos et al., 2020). It was an eco-friendly IWM approach, has gained prominence in organic agriculture and herbicide-resistant weed management (Wellhausen et al. 2018; De Cauwer et al. 2020). This method leverages pre-planting weed germination and subsequent elimination to minimize weed pressure during crop establishment. Its efficacy has been validated across diverse cropping systems, including wheat (Rasmussen, 2004), barley (Kanas et al., 2020b), corn (Gieske et al., 2016), rice (Cho et al., 2014) and several vegetables (De Cauwer et al., 2019), solidifying its role as a cornerstone of sustainable weed management (Merfield, 2013).

Post-tillage light irrigation is often applied to stimulate weed emergence, particularly in arid environments (Kanas et al., 2020a). The technique's success depends critically on two factors: Suitable temperatures and adequate soil moisture to maximize weed germination prior to crop planting. Although, seed dormancy also plays a role in this practice and breaking seed dormancy is a prerequisite for the implementation of this technique (Boddy et al., 2013). In rice paddies, this method effectively suppresses early-season weed emergence, especially for annual weeds reliant on seed reproduction (Travlos et al., 2020).

In Shanghai, China, regional climatic conditions—characterized by an average pre-transplantation temperature of 18 ~ 22 °C and seasonal rainfall of 180 ~ 250 mm during April and May. Local agricultural practices synergize with this technique: Rice fields are typically maintained as fallow fields or green manure plots (e.g., *Vicia faba*) during the pre-cultivation period. As Shanghai's cropland supports only a single annual rice-growing season, routine pre-planting tillage for fallow or green manure management inherently integrates the false-seedbed workflow without additional operational (i.e., pre-herbicides treatments) costs. Despite these advantages, a critical knowledge gap persists in optimizing the technique's implementation. The optimal interval between initial tillage and seedling elimination—a key determinant of weed control efficacy—remains poorly defined and likely varies with crop type, soil properties, and regional climate (De Cauwer et al., 2020). Furthermore, weeds not only serve as harmful organisms in farmland, but also have other ecological benefits in agroecosystems. Weeds enhance biodiversity, provide microhabitats for arthropods, and improve soil fertility through root and litter decomposition (Marshall et al., 2003), their incorporation into soil via tillage may alter soil microbial communities and physicochemical properties—critical drivers of crop productivity (Li et al., 2023; Huang et al., 2024). Despite the false-seedbed technique's weed control benefits, its effects on soil health parameters such as microbial diversity, organic C, and nutrient cycling remain unclear.

To address these, our study firstly aimed to identify the ideal induction time and tillage frequency for maximizing weed seed germination in Shanghai's rice agroecosystems. The second objective was to assess how different false-seedbed practices influence soil microbial diversity and physicochemical properties, ensuring weed management strategies align with agricultural sustainability. By integrating weed control efficacy with soil health assessments, this work provides a holistic understanding of the false-seedbed technique's trade-offs and opportunities for sustainable rice production.

MATERIALS AND METHODS

Field site location and experiment design

False-seedbed experiments were conducted consecutively before the rice-planting seasons of 2023 and 2024 at the Chonggu Experimental Station, Shanghai Academy of Agricultural Sciences (31.19° N, 121.20° E), located in Qingpu District, Shanghai, China. The soil type was Hydragric Anthrosols. The experimental fields had been under continuous rice (*Oryza sativa* L.) monoculture for over 5 yr, with increasing weed pressure annually. Dominant weed species included *Echinochloa crus-galli* (L.) P. Beauv., *Leptochloa chinensis* (L.) Nees, *Ammannia auriculata* Willd., and *Cyperus difformis* L. Prior to the experiment, the fields were plowed once and amended with green manure (*Vicia faba* L.), following standard local practices. A homogeneous field (~ 3000 m²) was divided into six randomized blocks (each ~ 500 m²) to test six treatments (Table 1): Induction 0 d and tillage once before rice sowing (CG0), induction 20 d and tillage once before rice sowing (CG20), induction 30 d and tillage once before rice sowing (CG30), induction 40 d and tillage once before rice sowing (CG40), induction 20

d and tillage once and then induction 20 d and plowing once before rice sowing (CG40-2), induction 0 d and herbicide treatment (30% bensulfuron-methyl [methyl 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoylmethyl]benzoate] + pretilachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(2-propoxyethyl)acetamide] oil dispersion, 450 g ai ha⁻¹) before rice sowing (CGH). The rotary tiller was used to plow the land at a depth of 15 ~ 20 cm and the detailed operational processes are listed in Table 1. Each block was independently irrigated and drained to prevent cross-contamination. During the induction period, the rice fields were maintained in an alternating state of wetting and drying. Once the soil became dry, extra irrigation was conducted; conversely, if the field plot accumulated as much water as possible, timely drainage was necessary to ensure that the weed seeds were induced to germinate. Weed germination was monitored weekly, and final weed density was recorded on 5 June for all treatments. Following weed assessment, fields were tilled by rotary tillage and prepared for water-direct-seeded rice cultivation. All post-treatment agronomic practices (e.g., irrigation, fertilization, pest management, etc.) followed standardized protocols to minimize confounding variables.

Table 1. Detailed operation process of the false-seedbed technique (2023 and 2024). CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

Treatment	Operation process in 2023 and 2024
CG20	15 May: Irrigation 5 June: Survey, tillage, and irrigation 7 June: Rice sowing 21 June: Survey
CG30	5 May: Irrigation 5 June: Survey, tillage, and irrigation 7 June: Rice sowing 21 June: Survey
CG40	25 April: Irrigation 5 June: Survey, tillage, and irrigation 7 June: Rice sowing 21 June: Survey
CG40-2	25 April: Irrigation 15 May: Survey, tillage, and irrigation 5 June: Survey, tillage, and irrigation 7 June: Rice sowing 21 June: Survey
CG0	5 June: Survey, tillage, and irrigation 7 June: Rice sowing 21 June: Survey
CGH	5 June: Survey, tillage, and irrigation 7 June: Rice sowing and herbicide application 21 June: Survey

Weed control efficacy assessment

Weed control efficacy was evaluated using two parameters: Total number of weeds induced during the false-seedbed phase and total number of weeds emerging 14 d post-sowing. Here, the four main weeds mentioned previously were counted, and the occurrence numbers of the four weeds were added to obtain the total number of weeds. Each treatment was randomly surveyed for the number of weed occurrences within a range of 0.5 m × 0.5 m, with 10 replicates. For CG40-2, surveys were conducted three times (an additional

investigation was conducted on the 20th day, 15 May), and the surveys were conducted twice for the rest of the treatments. The number of weeds induced before rice sowing was calculated as the sum of the results of the two surveys (15 May and 5 June) for CG40-2. After 14 d of rice sowing, the number of newly emerged weeds was investigated on 21 June. Total number of weed occurrences of CG0 after 14 d of rice cultivation was set as the control; therefore, the control efficacy could be calculated by the following formula: Control efficacy (%) = (Total number of weed occurrences of CG0 - Total number of weed occurrences of each treatment)/Total number of weed occurrences of CG0 × 100%.

Soil samples collection

Soil samples were collected 14 d post-sowing on 21 June, coinciding with the peak emergence of new weeds and the decomposition of pre-sowing induced weeds into the soil. At each treatment plot, soil cores (0 ~ 10 cm depth) were collected using a stainless-steel auger (5 cm diameter) at four randomized sampling points to account for spatial variability. From each point, two subsamples were collected, one sample was stored at 4 °C for physicochemical analyses; the other was flash-frozen in liquid nitrogen and stored at -80 °C for microbial community profiling. Samples were transported to the laboratory within 2 h of collection. Following sampling, targeted herbicide applications were performed to control residual weeds and ensure unimpeded rice growth, as false-seedbed treatments exhibited high residual weed pressure.

Soil physicochemical properties determination

Air-dried soil samples were gently crushed, sieved (< 2 mm), and analyzed for key physicochemical properties using standardized methods. The pH was measured according to the Chinese Standard (NY/T 1377-2007 Determination of Soil pH) using a pH meter (PHS-3C, INASE Scientific Instrument, Shanghai, China). Total N (TN) was determined using an automatic Kjeldahl nitrogen analyzer (KDN-19K, Shanghai Xianjian Instrument, Shanghai, China). Total P (TP) was determined using the alkali fusion-Mo-Sb anti-spectrophotometric method according to the standard procedure (HJ 632-201) of the Ministry of Environmental Protection of China. The total K (TK) was measured using the flame photometer method with a flame photometer (FP6410, Shanghai Huyueming Scientific Instrument, Shanghai, China). Total organic C (TOC) was measured via high-temperature catalytic oxidation ($K_2Cr_2O_7$ - H_2SO_4) using a TOC analyzer (BRAND Trading, Shanghai, China).

Soil DNA extraction and microbial community profiling

DNA extraction and quality control. Total genomic DNA was extracted from soil subsamples (stored at -80 °C) using the OMEGA Soil DNA Kit (M5635-02; Omega Bio-Tek, Norcross, Georgia, USA), following the manufacturer's protocol. The DNA concentration and purity were assessed using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), with absorbance ratios ($A_{260/280}$ and $A_{260/230}$) between 1.8 ~ 2.0 indicating high-quality DNA. Integrity was verified via 1% agarose gel electrophoresis, and samples were stored at -20 °C until further analysis.

16S rRNA gene amplification and sequencing. The hypervariable V3-V4 region of bacterial 16S rRNA genes was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Each primer pair included a unique 7 bp barcode for multiplexing. PCR reactions (25 µL) containing 12.5 µL 2× Taq Master Mix, 1 µL each primer (10 µM), 10 ng template DNA, and nuclease-free water. Thermocycling conditions were: 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s; and final extension at 72 °C for 10 min. Amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Nanjing Vazyme Biotechnology, Nanjing, China) and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, California, USA). Equimolar pools of amplicons were sequenced on the Illumina NovaSeq 6000 platform (NovaSeq 6000 SP Reagent Kit, 500 cycles; Shanghai Personal Biotechnology, China), generating paired-end 250 bp reads.

Bioinformatics processing. Amplicons were analyzed by paired-end sequencing using the Illumina platform (Personal Biotechnology Company, Shanghai, China). With minor modifications, microbiome bioinformatics was performed using QIIME 2 2019.4, in accordance with official tutorials (<https://docs.qiime2.org/2019.4/tutorials>). The quality of the raw high-throughput sequences was evaluated. The trim-paired primer sequence fragments and

mismatched primer sequences were eliminated using CutAdapt. The selected sequences were quality-filtered, denoised, merged, and chimeras were eliminated using the Divisive Amplicon Denoising Algorithm 2 (DADA2) techniques (Callahan et al., 2016). DADA2 deduces sequences and produces identical amplicon sequence variations (ASVs) that are identical. Only dereplication or grouping based on 100% similarity was performed for the DADA2. At that sequencing depth, each sample and its relative abundance predicted ASVs. Alpha-diversity metrics (Chao1, Faith_pd, Shannon, and Observed_species) and beta diversity metrics were estimated using the diversity plugin with samples rarefied to their respective sequences per sample. Taxonomy was assigned to the ASVs using the classify-sklearn naive Bayes taxonomy classifier in the feature-classifier (Bokulich et al., 2013) plugin against the SILVA Release 138.1 Database (<http://www.arb-silva.de>).

Statistical analysis

Differences in weed density and soil physicochemical properties across treatments were assessed using one-way ANOVA followed by Duncan's multiple range test ($\alpha = 0.05$) in SPSS Statistics 27.0 (IBM Corp., Armonk, New York, USA). Data normality and homogeneity of variance were verified using Shapiro-Wilk and Levene's tests, respectively.

The ASV table in QIIME 2 was used to calculate the microbial alpha-diversity parameters, including Chao1 richness, observed species, Shannon diversity index, and Simpson's index. Nonmetric multidimensional scaling (NMDS), based on Bray-Curtis distance and hierarchical clustering, was conducted using the Vegan package within the statistical software R-3.2.0 (R Foundation for Statistical Computing, Vienna, Austria) to examine the similarity and variation of community composition across different treatments. The ASV-level ranked abundance curves were generated to compare the richness and evenness of the ASVs among the samples.

Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using Jaccard metrics, Bray-Curtis metrics, and UniFrac distance metrics, and visualized via NMDS. The taxonomic composition and abundance were visualized using MEGAN and GraPhlAn. Venn diagram was generated to visualize the shared and unique ASVs among samples or groups using R package "VennDiagram," based on the occurrence of ASVs across samples/groups regardless of their relative abundance.

Co-occurrence network analysis was performed using the SparCC analysis. The pseudo-count value for the SparCC was set to 10^{-6} . The cutoff for the correlation coefficients was determined to be 70 using random matrix theory-based methods, as implemented in the R package RMThreshold. Based on the correlation coefficients, we constructed a co-occurrence network with nodes representing ASVs and edges representing the correlations between these ASVs. Canonical correlation analysis (CCA) was performed using the Vegan package, and the Monte Carlo Permutation test was used to evaluate whether the correlation between the response and explanatory variables was significant. The network was visualized using the R packages, iGraph and gGraph.

RESULTS

Weed control efficacy under different false-seedbed parameters

The data of 2023 and 2024 experiments did not show significant difference. The number of weeds induced before rice sowing varied significantly across treatments (Table 2). The CG0 (no induction) and CGH (herbicide control) exhibited the lowest weed densities ($30 \sim 35$ weeds 0.25 m^{-2}). In contrast, induction periods of 20 d (CG20) and 30 d (CG30) increased weed emergence to ~ 60 and ~ 90 weeds 0.25 m^{-2} . The longest induction periods (CG40 and CG40-2) resulted in the highest weed densities (~ 120 and ~ 115 weeds 0.25 m^{-2} , respectively). Notably, CG40 and CG40-2 showed nonsignificant difference in total number of weeds induced. Post-sowing weed control efficacy mirrored these trends (Table 3). The CGH achieved the highest control effect ($\sim 85\%$). Among false-seedbed treatments, CG40 and CG40-2 demonstrated superior control ($\sim 75\%$), outperforming shorter induction periods (CG20: $\sim 20\%$; CG30: $\sim 40\%$).

Table 2. Number of weeds induced under different treatments before rice sowing. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

		Numbers of induced weeds (0.25 m ²)									
		Echinochloa species		Leptochloa chinensis		Ammannia auriculata		Cyperus difformis		Total	
Treatments		2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
CG0		11.3 ± 4.03 ^d	10.5 ± 2.61 ^d	5.1 ± 1.45 ^d	5.4 ± 1.28 ^d	5.7 ± 2.69 ^c	5.0 ± 1.67 ^c	11.5 ± 1.96 ^c	8.9 ± 2.21 ^c	33.6 ± 4.18 ^d	29.8 ± 3.92 ^d
		20.7 ± 4.29 ^c	17.2 ± 3.46 ^c	7.9 ± 2.34 ^c	7.7 ± 2.05 ^c	15.5 ± 3.38 ^b	13.8 ± 3.12 ^b	19.5 ± 6.00 ^b	13.6 ± 3.64 ^b	63.6 ± 6.02 ^c	52.3 ± 6.99 ^c
CG30		27.7 ± 4.73 ^b	24.6 ± 4.43 ^b	14.0 ± 3.03 ^b	16.1 ± 1.92 ^b	22.2 ± 2.60 ^a	23.2 ± 2.48 ^a	25.1 ± 5.11 ^a	27.4 ± 4.18 ^a	89.0 ± 6.12 ^b	91.3 ± 5.06 ^b
		42.7 ± 3.58 ^a	44.4 ± 3.00 ^a	24.0 ± 2.83 ^a	26.9 ± 2.47 ^a	23.2 ± 5.29 ^a	24.8 ± 4.33 ^a	26.8 ± 2.27 ^a	28.7 ± 2.15 ^a	116.7 ± 5.68 ^a	124.8 ± 4.91 ^a
CG40-2		44.0 ± 2.79 ^a	45.8 ± 2.56 ^a	23.7 ± 3.93 ^a	24.0 ± 1.95 ^a	22.8 ± 3.60 ^a	22.2 ± 3.52 ^a	25.1 ± 4.11 ^a	25.2 ± 5.72 ^a	115.6 ± 6.55 ^a	117.2 ± 5.74 ^a
		11.1 ± 2.17 ^d	11.4 ± 1.20 ^d	7.0 ± 2.14 ^{cd}	6.5 ± 0.80 ^d	5.3 ± 1.79 ^c	5.7 ± 1.34 ^c	11.9 ± 3.91 ^c	11.9 ± 3.91 ^c	35.3 ± 4.29 ^d	35.3 ± 4.29 ^d

Table 3. Number of weeds induced under different treatments after rice sowing for 14 d. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

		Numbers of induced weeds (0.25 m ²)					Control efficacy (%)
		Echinochloa species	Leptochloa chinensis	Ammannia auriculata	Cyperus difformis	Total	
2023	CG0	44.7 ± 5.53 ^a	24.2 ± 2.89 ^a	23.9 ± 2.47 ^a	24.7 ± 2.72 ^a	117.5 ± 8.48 ^a	/
	CG20	31.5 ± 3.69 ^b	23.0 ± 3.61 ^a	21.6 ± 5.04 ^a	16.2 ± 3.03 ^b	92.3 ± 5.51 ^b	21.4 ± 4.69 ^d
	CG30	21.1 ± 4.37 ^c	9.7 ± 2.33 ^b	18.5 ± 3.38 ^b	18.0 ± 4.10 ^b	67.3 ± 7.71 ^c	42.7 ± 6.56 ^c
	CG40	12.6 ± 1.80 ^d	5.8 ± 1.54 ^c	5.2 ± 1.94 ^c	5.4 ± 1.74 ^{cd}	29.0 ± 4.40 ^d	75.3 ± 3.75 ^b
	CG40-2	12.2 ± 2.89 ^d	6.7 ± 2.28 ^c	5.4 ± 2.15 ^c	7.4 ± 2.37 ^c	31.7 ± 4.38 ^d	73.0 ± 3.73 ^b
	CGH	5.0 ± 1.41 ^e	5.4 ± 1.62 ^c	3.6 ± 1.11 ^c	3.2 ± 1.33 ^d	17.2 ± 2.71 ^e	85.4 ± 2.31 ^a
2024	CG0	44.2 ± 4.56 ^a	26.4 ± 4.45 ^a	25.8 ± 2.23 ^a	25.6 ± 4.15 ^a	122.0 ± 6.84 ^a	/
	CG20	31.9 ± 3.51 ^b	24.4 ± 2.15 ^a	23.3 ± 2.49 ^a	17.4 ± 1.80 ^b	97.0 ± 5.67 ^b	20.5 ± 4.65 ^d
	CG30	22.1 ± 2.42 ^c	10.8 ± 0.87 ^b	18.9 ± 1.92 ^b	18.8 ± 2.09 ^b	70.6 ± 3.27 ^c	42.1 ± 2.68 ^c
	CG40	13.2 ± 1.47 ^d	6.0 ± 1.18 ^c	6.3 ± 1.80 ^c	5.8 ± 1.17 ^{cd}	31.3 ± 3.35 ^d	74.3 ± 2.74 ^b
	CG40-2	12.2 ± 2.89 ^d	6.5 ± 1.43 ^c	4.9 ± 1.51 ^c	7.0 ± 1.34 ^c	30.6 ± 3.72 ^d	74.9 ± 3.05 ^b
	CGH	4.8 ± 0.87 ^e	4.8 ± 1.08 ^c	4.1 ± 1.04 ^c	3.0 ± 0.77 ^d	16.7 ± 2.05 ^e	86.3 ± 1.76 ^a

Soil physicochemical properties

Soil properties varied significantly among treatments (Table 4). The CGH displayed worst quality compared to the false-seedbed treatments from the view of pH, TN, TP and TOC. The pH values ranged from 7.29 ~ 7.82 in false-seedbed treatments with nonsignificant difference, significantly higher than that of CGH (7.06 and 7.20 in 2023 and 2024, respectively). For TN, the highest values were presented in CG40 (2.2 g kg⁻¹) and CG40-2 (2.1 g kg⁻¹), surpassing shorter induction periods (1.26 ~ 1.58 g kg⁻¹); while TP was reduced with longer induction, with CG40 and CG40-2 (1.21 ~ 1.27 g kg⁻¹) lower than that of CG0 ~ CG30 in 2023 (1.46 ~ 1.57 g kg⁻¹) and showed

nonsignificant difference in 2024; TK was also reduced with longer induction, with CG40 and CG40-2 ($18.34 \sim 18.39 \text{ g kg}^{-1}$) lower than that of CG20 $\sim$ CG30 in 2024 ($19.60 \sim 19.95 \text{ g kg}^{-1}$) and did not show significant difference in 2023; TOC was significantly increased with induction time, peaking in CG40-2 and CG40, followed by CG30, CG20, CGH and CG0.

Table 4. Effect of different false-seedbed technique treatments on soil quality improvement after rice sowing for 14 d. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

Treatments	pH		Total N		Total P		Total K		Total organic C	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
	g kg ⁻¹		g kg ⁻¹		g kg ⁻¹		g kg ⁻¹		g kg ⁻¹	
CG0	7.37 ± 0.03 ^a	7.60 ± 0.07 ^a	1.26 ± 0.08 ^c	1.28 ± 0.04 ^c	1.57 ± 0.08 ^a	1.38 ± 0.02 ^a	19.15 ± 0.46 ^a	19.29 ± 0.32 ^{ab}	9.42 ± 2.16 ^b	10.44 ± 0.83 ^c
CG20	7.45 ± 0.02 ^a	7.70 ± 0.22 ^a	1.35 ± 0.16 ^c	1.39 ± 0.03 ^{bc}	1.46 ± 0.06 ^a	1.31 ± 0.10 ^a	18.18 ± 0.32 ^{ab}	19.95 ± 0.49 ^a	12.67 ± 1.29 ^b	13.87 ± 1.20 ^b
CG30	7.40 ± 0.02 ^a	7.82 ± 0.17 ^a	1.58 ± 0.08 ^{bc}	1.58 ± 0.07 ^b	1.51 ± 0.05 ^a	1.25 ± 0.05 ^a	17.89 ± 0.43 ^{ab}	19.60 ± 0.21 ^a	12.40 ± 2.50 ^b	14.86 ± 0.76 ^b
CG40	7.33 ± 0.03 ^a	7.61 ± 0.04 ^a	2.21 ± 0.16 ^a	2.19 ± 0.08 ^a	1.27 ± 0.03 ^{bc}	1.23 ± 0.05 ^a	17.75 ± 0.71 ^{ab}	18.39 ± 0.35 ^b	17.92 ± 0.68 ^a	22.61 ± 0.64 ^a
CG40-2	7.29 ± 0.07 ^a	7.61 ± 0.04 ^a	2.11 ± 0.10 ^a	2.09 ± 0.02 ^a	1.21 ± 0.13 ^{bc}	1.24 ± 0.02 ^a	18.12 ± 0.33 ^{ab}	18.34 ± 0.30 ^b	22.04 ± 3.57 ^a	21.95 ± 0.33 ^a
CGH	7.06 ± 0.08 ^b	7.23 ± 0.07 ^b	1.85 ± 0.06 ^{ab}	1.26 ± 0.04 ^c	1.11 ± 0.06 ^c	0.86 ± 0.03 ^b	16.80 ± 0.83 ^b	17.49 ± 0.44 ^c	12.43 ± 1.06 ^b	13.77 ± 0.94 ^b

Soil microbial community change

In total, 215 187 raw sequences were obtained across all samples, ranging from 59 668 to 136 799 sequences per sample, by sequencing the V3-V4 regions of the 16S rRNA of the soil samples. In total, 1 202 193 high quality sequences were obtained after quality control. The raw sequences obtained across all samples were sequenced against the V3-V4 regions of 16S rRNA of the rhizosphere soil flora. A total of 36 309 ASVs were detected in all samples. The number of unique ASVs in all the samples was 1040.

Across all soil samples, bacterial communities at the phylum level were dominated by Proteobacteria, which accounted for 41.76% $\sim$ 46.57% of the relative abundance, followed by Acidobacteria at 9.11% $\sim$ 15.24% and Gemmatimonadetes at 7.44% $\sim$ 10.96% (Figure 1). The mean relative abundance of the bacterial communities (e.g., Proteobacteria, Gemmatimonadetes, Bacteroidetes, Chlorobi and Actinobacteria) at the phylum level did not change significantly among the treatments. For Acidobacteria and Nitrospirae, the mean relative abundance was significantly higher in CGH than any other treatment. For the rest of the bacterial communities, Chloroflexi and WS3 was significantly higher in CG0; Verrucomicrobia was significantly higher in CG20. Therefore, the bacterial communities at the phylum level were different among all the treatments.

Alpha diversity of the bacterial communities was evaluated using multiple indices (Figure 2). The Chao1 (indicating richness), Faith_pd (indicating diversity based on evolution), Shannon (indicating diversity), and Observed_species (indicating richness) indices showed that the diversity and relative abundance of bacteria in CG40-2 were significantly higher than those in CGH. In addition, the Faith_pd index of the CG20 group was significantly higher than the CGH group.

The beta diversity of the bacteria is represented by NMDS ordinations with stress values of 0.12 (Figure 3a). For the false-seedbed technique groups (CG20, CG30, CG40, and CG40-2), the scatter plots usually clustered toward CG0. The confidence ellipses formed by the scatter points of the respective false-seedbed technique groups overlapped with the confidence ellipses of the control group (CG0). However, there was no overlap between the confidence ellipses of CGH and CG0. The confidence ellipse of CGH was independent of the confidence ellipse of CG0. Hierarchical cluster analysis revealed no obvious clustering trend in the soil samples.

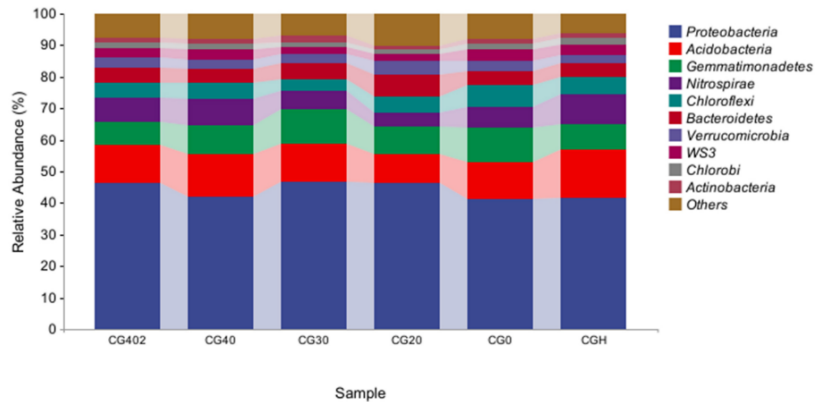


Figure 1. Relative abundance of major bacteria by phylum from soil samples in response to different parameters of the false-seedbed technique. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

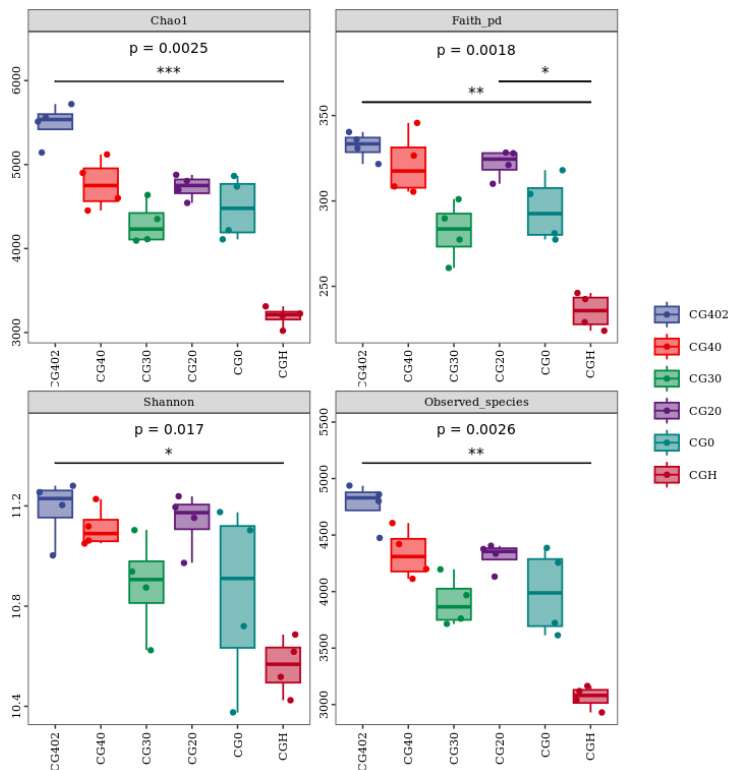


Figure 2. Comparison of alpha-diversity of bacteria from soil samples using the false-seedbed technique. **p < 0.01; *p < 0.05. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

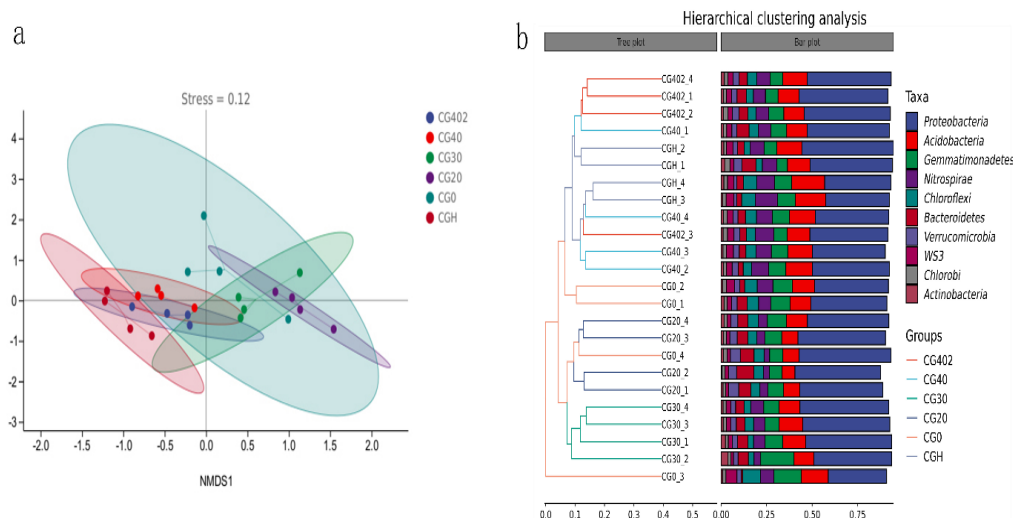


Figure 3. Comparison of beta-diversity of bacteria from soil samples using the false-seedbed technique. (a) Beta-diversity of microbial communities shown by nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis distance. Stress: < 0.05, excellent < 0.10 = good, < 0.20, usable; and > 0.20, not acceptable. (b) Beta diversity of microbial communities, as shown by hierarchical clustering. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

The top 10 species at the phylum level are shown (Figure 3b). Linear discriminant analysis effect size (LEfSe) was used to study the differences in microorganisms at various classification levels among the different treatments, which were represented using a cladogram and distribution histogram (Figure 4). No enriched differentially expressed species were detected in the CG40 group. When the linear discriminant analysis (LDA) score was greater than 2, 62 bacterial species were detected in the microbial communities, which were significantly different among all the treatments ($p < 0.05$).

Correlations of soil properties and microbial communities

The canonical correlation analysis (CCA) was used to explore the influence of soil properties and to identify the factors affecting microbial community structure (Figure 5a). The bacterial community structure was affected by pH, TOC, TP, TK, and TN soil content. The first and second ordination axes explained 12.15% and 15.01% of the overall variation, respectively. The length of the arrow was used to measure the degree of correlation between environmental factors and microbial community distribution. The TOC and TP showed greater correlations than pH, TK, and TN. In addition, pH, TP, and TK were positively and negatively correlated with TN and TOC, respectively.

Pearson's correlation coefficient was used to analyze the correlation between dominant microbial phyla and soil properties (Figure 5b). GOUTA19 expression negatively correlated with TOC. *Thiobacillus* was negatively correlated with TOC and TN and positively correlated with TP. Unidentified_Ellin6529 negatively correlated with TP. Unidentified_Bacteroidales were negatively correlated with the TOC. Unidentified_Gemm-5 was positively correlated with the soil pH. The 4-29, unidentified_Bacteria and unidentified_Ignavibacteriaceae were negatively correlated with TN. Unidentified_Gemm-3 was positively correlated with soil pH and TP. Unidentified_Sva0725 was negatively correlated with TK and TP, and positively correlated with TN. Unidentified_SC-I-84, unidentified_MVS-40, unidentified_Alcaligenaceae, unidentified_Deltaproteobacteria, unidentified_BPC076, unidentified_Proteobacteria, and unidentified_11-24 were negatively correlated with the TN and TOC levels.

The correlation matrix heat maps of soil properties with potential bacteria are shown in Figure 5c. Only TOC was significantly correlated with the bacterial species. The correlations among the five soil property parameters followed those shown in Figure 5.

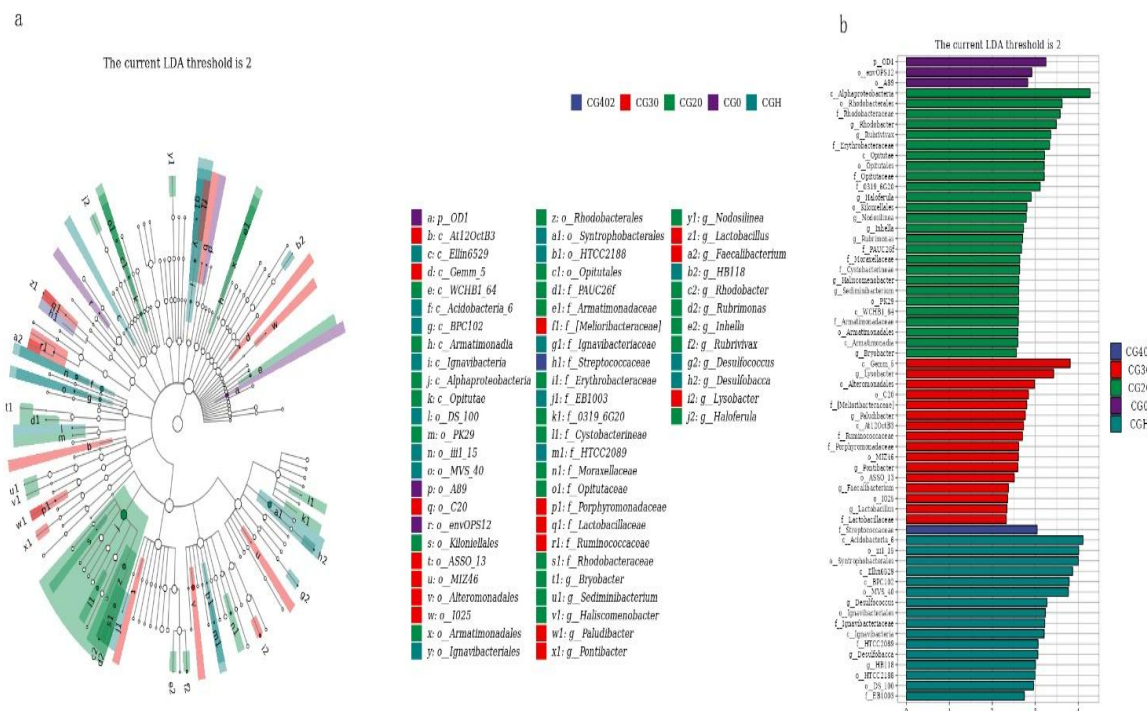


Figure 4. Cladogram (a) and distribution histogram (b) based on LEfSe analysis of soil sample from soil samples of the false seedbed technique. The cladograms were used to depict the hierarchical taxonomic distribution of the marker species that were significantly enriched in the samples of each community. Taxonomic levels from phylum to genera are represented by circles radiating outward from the center. The diameter of each small circle corresponds to the relative abundance size, and each small circle at different taxonomic levels reflects a single classification at that level. Significantly different species were colored following the original hue of the group, whereas species with no notable variations were consistently white. The distribution histograms show significantly enriched species at various classification levels and their importance within each group. The histogram bar length corresponds to the LDA score, which measures the degree of influence of significantly different species between various groups. The colors of the histogram correspond to the corresponding sample groups. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

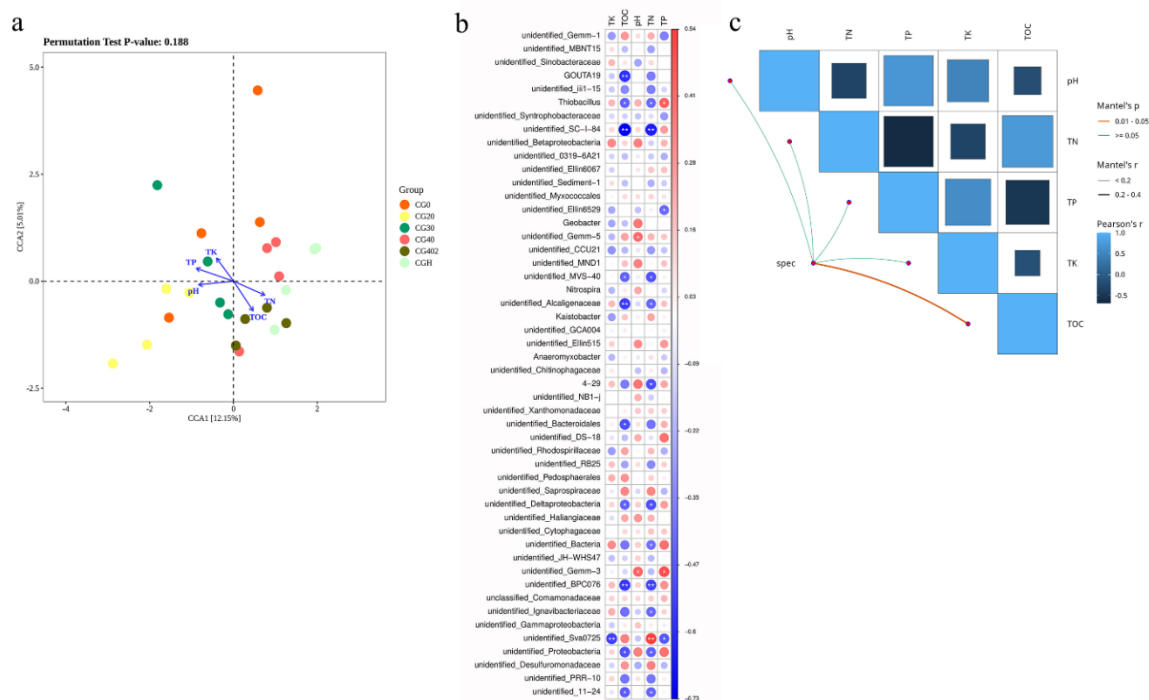


Figure 5. Correlations of soil properties and microbial communities. (a) Canonical correlation analysis (CCA) of bacterial composition and corresponding soil physicochemical properties. TN: Total N; TP: total P; TK: total K; TOC: total organic C. (b) Pearson's correlation coefficient analysis between dominant microbial phyla and soil properties. A positive correlation is shown in red; a negative correlation is shown in blue; and the depth of color indicates the strength of the correlation. The asterisk (*) indicates a significant correlation ($p < 0.05$) between microbial species and soil properties. (c) Correlations between environmental and plant variables and potentially beneficial bacteria. Colors indicate the correlation types. The edge width corresponds to the Mantel's r value determined by the linear mixed-effects model. Solid and dashed lines denote significant and nonsignificant correlations, respectively. Pairwise comparisons of factors are shown in the triangle, with a color gradient denoting Pearson's correlation coefficient. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CG0: Induction at 0 d and tillage once before rice sowing; CG20: induction for 20 d and tillage once before rice sowing; CG30: induction for 30 d and tillage once before rice sowing; CG40: induction 40 d and tillage once before rice sowing; CG40-2: induction for 20 d and tillage once and then induction for another 20 d and plowing once before rice sowing; CGH: induction for 0 d and herbicide treatment (30% bensulfuron-methyl+pretilachlor oil dispersion, 450 g ai ha⁻¹) before rice sowing.

DISCUSSION

Weed-control effect of the false-seedbed technique

The false-seedbed technique offers a sustainable alternative to chemical herbicides, particularly in systems challenged by herbicide resistance or organic farming mandates. Unlike stale seedbeds, which rely on nonselective herbicides like glyphosate, the false-seedbed method employs mechanical weed elimination, making it environmentally favorable (Gazoulis et al., 2023). Previous studies in upland crops reported variable success: 65%-73% weed suppression in Chinese cabbage and soybean, and up to 80% reduction in *Poa trivialis* and *Vulpia myuros* (Cho et al., 2014; De Cauwer et al., 2019; Jensen, 2019). Our findings align with these results, demonstrating a 73%-75% control efficacy in rice paddies (Table 3), which was lower compared to herbicide treatments (CGH: 85.4%).

In Shanghai, the technique was optimized using pre-existing practices that green manure cultivation and fallow field management required no additional costs or machinery. Longer induction periods (40 d) maximized pre-sowing weed germination (Table 2), reducing post-sowing weed pressure. This aligns with studies showing that 4 ~ 6 wk of induction significantly depletes the soil seedbank (Lonsbary et al., 2003). Notably, annual weeds (*Echinochloa* spp., *L. chinensis*, *A. auriculata*, and *C. difformis*) that rely entirely on seed reproduction were effectively controlled through induced germination (Table 2), demonstrating the technique's targeted efficacy against obligate seed-propagated species.

While the false-seedbed alone achieved 75% efficacy, combining it with herbicides (e.g., CGH) enhanced control to 85%. Similar synergies were reported in soybean and cucumber systems, where integrated approaches reduced weed density by 95% (Kanas et al., 2020a). Another report also confirmed that a stale-seedbed combined with herbicides was a superior integrated weed management tool compared to conventional weed-management practices for machine-harvested cucumbers (*Cucumis sativus* L.) (Lonsbary et al., 2003). However, reliance on herbicides contradicts the technique's eco-friendly premise. Thus, we advocate its use as a pre-planting auxiliary strategy to minimize herbicide dependency, particularly in regions like Shanghai, where policy subsidies support green manure integration.

The lack of difference between CG40 and CG40-2 (Table 3) suggests that a single 40 d induction suffices, avoiding redundant tillage. This aligns with Greek practices, where false seedbeds are subsidized for rice cultivation (Kanas et al., 2020b). In Shanghai, the technique's alignment with routine pre-sowing tillage eliminates operational overhead, making it economically viable for smallholder farmers. Further research should refine parameters (e.g., tillage depth, machinery type) across diverse rice systems. For instance, harrowing *versus* hoeing may differentially affect weed seed burial and germination. Additionally, regional trials are needed to adapt induction periods to local climates—shorter in tropical zones with rapid weed cycles, longer in temperate regions. The false-seedbed technique aligns with global shifts toward sustainable agriculture. Its low cost (~ 75% efficacy without herbicides) and minimal C footprint make it ideal for organic certification programs. Policymakers should incentivize adoption through subsidies for green manure seeds or training in mechanical weed management, as seen in Greece and parts of Asia (Schutte et al., 2021).

Ecological effect of the false-seedbed technique

Longer induction periods (40 d) under the false-seedbed technique significantly enhanced total N (TN) and total organic C (TOC) compared to shorter induction periods (CG0 ~ CG30; Table 4). This aligns with a previous report (Gu et al., 2019), which attributed similar trends to organic matter accumulation from decomposed weed biomass. The prolonged presence of weeds likely increased root exudates and litter inputs, fostering microbial activity and nutrient retention while reducing N leaching (Huang et al., 2024). In contrast, total P (TP) declined with longer induction, likely due to leaching during Shanghai's rainy pre-sowing season (Gu et al., 2019). Notably, herbicide treatment (CGH) reduced soil pH (7.06 vs. 7.29 ~ 7.45 in other treatments), mirroring acidification effects observed in atrazine-treated soils (Ayansina and Oso, 2006), and lowered total K (TK), possibly due to altered cation exchange dynamics.

Soil microbial diversity typically represents changes in soil quality and reflects the overall dynamic changes in microbial communities. The relative abundance of major bacteria by phylum in the soil samples did not change significantly (Figure 1), indicating that the false-seedbed technique with different experimental parameters had different effect on the relative abundance of major bacteria. Further alpha-diversity analysis revealed that the herbicide treatment significantly reduced the diversity and richness of bacteria compared with the false-seedbed technique treatments (Figure 2). The bacterial population was reduced by 27.3% when mesosulfuron + iodosulfuron mixture was applied to wheat, following application of pendimethalin followed by imazethapyr in the preceding soybean crop (Singh et al., 2015). Herbicide atrazine and atrazine + metolachlor treatments at both the recommended and 1.5-fold recommended rates resulted in decreased microbial counts, and higher concentrations of herbicide treatments resulted in much lower microbial counts compared with soils treated with the recommended herbicide doses (Ayansina and Oso, 2006). There is consensus that herbicides harm soil microorganisms. These effects of herbicides on microorganisms may reduce the performance of critical soil functions such as organic matter decomposition, N fixation, and phosphate solubilization, which support soil health, plant growth, and crop productivity (Singh et al., 2015).

The nonmetric multidimensional scaling (NMDS) analysis revealed that the herbicide treatment caused a

significant deviation compared to the false-seedbed technique treatments, whose scatter points were all in the confidence ellipses of the control group (Figure 3a). These results indicate that the induction time and tillage frequency of the false-seedbed technique were not the main factors influencing soil bacteria, whereas herbicide treatments did. Hierarchical cluster analysis revealed that CG0, CG20, and CG30 displayed greater clustering similarity, whereas CGH, CG40, and CG40-2 formed different clusters. This may be because the hierarchical cluster differences among the soil samples were determined by the number of newly emerged weeds. As shown in Table 3, weed numbers in CGH, CG40, and CG40-2 were significantly lower than those in CG0, CG20, and CG30. Fewer weeds that rot in the soil affect the hierarchical cluster, and detailed mechanisms should be explored. Similarly, bacterial species that were significantly different among the treatments did not show an obvious trend in terms of induction time and tillage frequency (Figure 3b). Changes in the diversity and richness of bacteria can lead to the disturbance of chemical and biological processes in agroecosystems and lead to the impairment of the soil nutrient balance (Nannipieri et al., 2003). Previous studies have mainly focused on the effect of herbicides on the richness of soil bacteria, whereas this study revealed changes in the richness, diversity, and structure of soil bacteria between the false-seedbed technique and the traditional herbicide treatment. The differences in soil physicochemical properties and soil microbial communities collectively demonstrated that the false-seedbed technique was beneficial for improving soil quality and promoting the health of microbial communities compared with herbicide treatment.

The correlation between soil physicochemical properties and microbial composition using the false-seedbed technique has not been elucidated. For the five soil physicochemical properties, the pH, TP and TK showed positive correlation effect and negative correlation effect to TN and TOC (Figure 5a). The corresponding soil physicochemical properties and related soil microbial species were presented in Figure 5b. Microbial species 1, 10, 2, 12, and 4 exhibited complex relationships with TK, TOC, pH, TN, and TP. This indicates that the changes in TOC and TN content were more complicated than the changes in TK, TP, and soil pH. Figure 5c strengthens this point by showing that TOC and microbial species are significantly correlated. Previous studies have reported that soil microorganisms play critical roles in soil organic matter decomposition, nutrient availability, and cycling (Dominati et al., 2010; Huang et al., 2024), whereas other studies have shown that soil physicochemical properties are important factors that affect soil microbial communities (Johansson et al., 2004; Singh et al., 2021). Therefore, the relationships between the soil physicochemical properties and soil microorganisms are mutual, complex, and dynamic. The correlation between soil physicochemical properties and microbial composition presented in this study provided an inspirational case to understand their relationships. More implementation scenarios of the false-seedbed technique are needed to obtain a comprehensive understanding.

CONCLUSIONS

This study demonstrates that a 40-d induction period with a single pre-sowing tillage optimizes the false-seedbed technique for rice cultivation, achieving ~ 75% weed control efficacy while enhancing soil health. Key advantages over herbicide-dependent methods include: Soil quality improvement, avoidance of herbicide impacts and high cost-effectiveness. Further research is needed to explore the best combination of the false-seedbed technique and chemical weed control to enhance weed management performance, as well as the interaction between weeds and soil in various crop cultivation systems. In summary, the false-seedbed technique balances efficacy, ecology, and economy, positioning it as a cornerstone for sustainable weed management in rice agroecosystems.

Author contribution

Conceptualization: J-P.F., Z-H.T. Methodology: J-P.F., Z-H.T. Software: G-H.Y., Y.G. Validation: G-H.S. Investigation: J-P.F., G-H.Y., Y.G. Data curation: G-H.S. Writing-original draft: J-P.F., G-H.S. Writing-review & editing: J-P.F., Z-H.T. Funding acquisition: Z-H.T. All co-authors reviewed the final version and approved the manuscript before submission.

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References

- Ayansina, A.D.V., Oso, B.A. 2006. Effect of two commonly used herbicides on soil microflora at two different concentrations. *African Journal of Biotechnology* 5(2):129-132.
- Boddy, L.G., Bradford, K.J., Fischer, A.J. 2013. Stratification requirements for seed dormancy alleviation in a wetland weed. *PLOS ONE* 8(9):e71457. doi:10.1371/journal.pone.0071457.
- Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., et al. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* 10(1):57-59. doi:10.1038/Nmeth.2276.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7):581-583. doi:10.1038/Nmeth.3869.
- Cho, J.-R., Ok, J.-h., Lee, B.-M., An, N.-H., Han, E.-J. 2014. Possibility of organic weed control by false- and stale-seedbed in upland crop. *Weed & Turfgrass Science* 3(3):215-220. doi:10.5660/WTS.2014.3.3.215.
- De Cauwer, B., Delanote, L., Devos, M., De Ryck, S., Reheul, D. 2020. Optimisation of weed control in organic processing spinach (*Spinacia oleracea* L.): Impacts of cultivar, seeding rate, plant spacing and integrated weed management strategy. *Agronomy* 11(1):1-18. doi:10.3390/agronomy11010053.
- De Cauwer, B., De Cuyper, T., De Ryck, S., Delanote, L., DeWaele, K., Willekens, K., et al. 2019. Reduction in field emergence and seedbank density of *Galinsoga quadriradiata* and other weeds after contrasting false seedbed strategies in organic vegetable fields. *Weed Research* 59(4):265-278. doi:10.1111/wre.12363.
- Dominati, E., Patterson, M., Mackay, A. 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological Economics* 69(9):1858-1868. doi:10.1016/j.ecolecon.2010.05.002.
- Fang, J.P., Zhang, Y.H., Liu, T.T., Yan, B.J., Li, J., Dong, L.Y. 2019. Target-site and metabolic resistance mechanisms to penoxsulam in barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) *Journal of Agricultural and Food Chemistry* 67(29):8085-8095. doi:10.1021/acs.jafc.9b01641.
- Gazoulis, I., Kanatas, P., Antonopoulos, N., Tataridas, A., Travlos, I. 2023. False seedbed for agroecological weed management in forage cereal-legume intercrops and monocultures in Greece. *Agronomy* 13(1):123. doi:10.3390/agronomy13010123.
- Gieske, M.F., Wyse, D.L., Durgan, B.R. 2016. Spring- and fall-seeded radish cover-crop effects on weed management in corn. *Weed Technology* 30(2):559-572. doi:10.1614/Wt-D-15-00023.1.
- Gu, X., Cen, Y., Guo, L.Y., Li, C.H., Yuan, H., Xu, Z.W., et al. 2019. Responses of weed community, soil nutrients, and microbes to different weed management practices in a fallow field in Northern China. *PeerJ* 7:e7650. doi:10.7717/peerj.7650.
- Huang, Z.C., Bian, F.Y., Wang, Z.G., Zhu, J.R., Zhang, X.P., Wang, J., et al. 2024. Microorganisms facilitated the saline-alkali soil remediation by biochar: Soil properties, microbial communities, and plant responses. *Land Degradation & Development* 35(11):3567-3578. doi:10.1002/ldr.5152.
- Iwakami, S., Hashimoto, M., Matsushima, K., Watanabe, H., Hamamura, K., Uchino, A. 2015. Multiple-herbicide resistance in *Echinochloa crus-galli* var. *formosensis*, an allohexaploid weed species, in dry-seeded rice. *Pesticide Biochemistry and Physiology* 119:1-8. doi:10.1016/j.pestbp.2015.02.007.
- Jensen, P.K. 2019. Use of integrated weed management tools in crop rotations with grass seed production. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 69(3):209-218. doi:10.1080/09064710.2018.1530295.
- Johansson, J.F., Paul, L.R., Finlay, R.D. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology* 48(1):1-13. doi:10.1016/j.femsec.2003.11.012.
- Juliano, L.M., Casimero, M.C., Llewellyn, R. 2010. Multiple herbicide resistance in barnyardgrass (*Echinochloa crus-galli*) in direct-seeded rice in the Philippines. *International Journal of Pest Management* 56(4):299-307. doi:10.1080/09670874.2010.495795.
- Kanatas, P.J., Travlos, I.S., Gazoulis, J., Antonopoulos, N., Tsekoura, A., Tataridas, A., et al. 2020b. The combined effects of false seedbed technique, post-emergence chemical control and cultivar on weed management and yield of barley in Greece. *Phytoparasitica* 48(1):131-143. doi:10.1007/s12600-020-00783-x.
- Kanatas, P., Travlos, I., Papastyliou, P., Gazoulis, I., Kakabouki, I., Tsekoura, A. 2020a. Yield, quality and weed control in soybean crop as affected by several cultural and weed management practices. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 48(1):329-341. doi:10.15835/nbha48111823.
- Li, P., Yan, Y., Li, C., Tang, W.G., Xiong, Z.Q., Tian, Y.H., et al. 2023. Response of rice growth to soil microorganisms and soil properties in different soil types. *Agronomy Journal* 115(1):197-207. doi:10.1002/agj2.21239.
- Lonsbary, S.K., O'Sullivan, J., Swanton, C.J. 2003. Stale-seedbed as a weed management alternative for machine-harvested cucumbers (*Cucumis sativus*). *Weed Technology* 17(4):724-730. doi:10.1614/Wt02-123.
- Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R., Ward, L.K. 2003. The role of weeds in supporting biological diversity within crop fields. *Weed Research* 43(2):77-89. doi:10.1046/j.1365-3180.2003.00326.x.
- Merfield, C. 2013. False and stale seedbeds: The most effective non-chemical weed management tools for cropping and pasture establishment. Available at <https://www.bhu.org.nz/future-farming-centre> (accessed 15 February 2025).
- Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G. 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54(4):655-670. doi:10.1046/j.1351-0754.2003.0556.x.

- Oerke, E.C. 2005. Crop losses to pests. *The Journal of Agricultural Science* 144(1):31-43. doi:10.1017/s0021859605005708.
- Oerke, E.C., Dehne, H.W. 2004. Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection* 23(4):275-285. doi:10.1016/j.cropro.2003.10.001.
- Rasmussen, I.A. 2004. The effect of sowing date, stale seedbed, row width and mechanical weed control on weeds and yields of organic winter wheat. *Weed Research* 44(1):12-20. doi:10.1046/j.1365-3180.2003.00367.x.
- Riar, D.S., Tehranchian, P., Norsworthy, J.K., Nandula, V., McElroy, S., Srivastava, V., et al. 2017. Acetolactate synthase-inhibiting, herbicide-resistant rice flatsedge (*Cyperus iria*): Cross-resistance and molecular mechanism of resistance. *Weed Science* 63(4):748-757. doi:10.1614/ws-d-15-00014.1.
- Schutte, B.J., Sanchez, A.D., Beck, L.L., Idowu, O.J. 2021. False seedbeds reduce labor requirements for weeding in chile pepper. *HortTechnology* 31(1):64-73. doi:10.21273/horttech04732-20.
- Singh, P., Sarathambal, C., Kewat, M.L., Singh, V.P. 2015. Conservation tillage and weed management effect on soil microflora of soybean-wheat cropping system. *Indian Journal of Weed Science* 47(4):366-370.
- Singh, D., Sharma, P., Kumar, U., Daverey, A., Arunachalam, K. 2021. Effect of forest fire on soil microbial biomass and enzymatic activity in oak and pine forests of Uttarakhand Himalaya, India. *Ecological Processes* 10(1):29. doi:10.1186/s13717-021-00293-6.
- Travlos, I., Gazoulis, I., Kanatas, P., Tsekoura, A., Zannopoulos, S., Papastylianou, P. 2020. Key factors affecting weed seeds' germination, weed emergence, and their possible role for the efficacy of false seedbed technique as weed management practice. *Frontiers in Agronomy* 2:1. doi:10.3389/fagro.2020.00001.
- Wellhausen, C., Ulber, L., Rissel, D. 2018. Investigation of crop management strategies for control of herbicide-resistant blackgrass (*Alopecurus myosuroides*). p. 82-86. In 28th German Conference on Weed Biology and Weed Control, Braunschweig, Germany. 27 February-1 March. Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Braunschweig, Germany.