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INVESTIGATING FACTORS INFLUENCING SOIL CARBON SEQUESTRATION IN CORN CULTIVATION USING THE TAGUCHI METHOD

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Abstract—With the acceleration of climate change in recent years, enhancing the carbon sequestration capacity of agricultural soil has become an important strategy for achieving sustainable agriculture and carbon neutrality. This study aimed to investigate the effects of multiple experimental factors on the soil carbon fixation efficiency of corn cultivation using the Taguchi method of experimental design. The following factors were investigated: amount of fertilizer, amount of irrigation, as well as soil supplementation with Rhodopseudomonas sp. strain AKW01 and Rhodoplanes sp. strain AKW02. We designed an L9(3^4) orthogonal array with three levels for each factor, and conducting nine sets of experimental plantings for each element in the array. Various outcomes were measured during different growth stages, including soil pH, soil electrical conductivity, soil organic carbon (SOC); as well as the height, chlorophyll content as determined using a Soil Plant Analysis Development (SPAD) meter, fresh weight, and dry weight of plant matter. The results of this study showed that the addition of the AKW01 strain significantly improved crop growth and carbon sequestration and was the primary factor affecting crop performance among the experimental factors studied, with a total variance contribution of 41.55%. Another significant factor was irrigation water amount, with a total variance contribution of 35.78%. Crop performance was optimal when the addition of the AKW01 strain was at 10 mL/plant, which led to a mean height of 138.1 cm, SPAD of 29.8, SOC increase of 0.01%, and fresh and dry weights of 95.38 g and 48.03 g, respectively.

Keywords—Carbon sequestration, Purple non-sulfur bacteria, Soil management, Sustainable agriculture, Taguchi experimental design method

I. INTRODUCTION

The frequency and intensity of extreme weather events have continued to rise in recent years due to increasingly severe global climate change, highlighting the importance of carbon sequestration technologies on an international level [1,2]. International initiatives, such as the United Nations Framework Convention on Climate Change (UNFCCC) and the Paris Agreement, emphasize the need to enhance natural carbon sequestration capacities as a means toward achieving net-zero emissions targets [3]. According to research by the Intergovernmental Panel on Climate Change (IPCC), soil is the second-largest carbon reservoir after the ocean [4]. If the organic carbon content of soil can be improved through appropriate agricultural management measures, this can potentially transform carbon sink resources. The development of soil management methods with carbon fixation potential has become a focal point in policy and academic fields. For example, Taiwan's 2050 Net-Zero Carbon Emissions Blueprint includes soil carbon sinks as a core structure of its agricultural carbon reduction strategy [5].

Agricultural ecosystems play a considerable role in the global carbon cycle, with soil being a key carbon reservoir. In recent years, both academic and practical fields have increasingly recognized that adjusting soil management strategies can help reconcile food production with carbon fixation goals, thereby achieving sustainable agriculture [6]. Corn, a major food crop, is characterized by its fast growth and high yield, makes it an attractive candidate for soil carbon sink strategies [7]. In other words, both crop yield and soil carbon accumulation can potentially be improved if the management of corn cultivation can be optimized.

Current agricultural management practices, such as fertilization, irrigation, and the application of microbial materials, have substantial impacts on crop growth and soil carbon dynamics. However, these factors often exhibit complex interactions, making it challenging for single-factor assessments to accurately reflect actual field performance [8]. Therefore, employing a systematic approach to comprehensively analyze multi-factor combinations will aid in identifying the best strategies that balance carbon fixation benefits with production efficiency.

In recent years, an increasing number of studies have focused on the role of microbial materials in the soil carbon cycle. Among these, purple non-sulfur bacteria (PNSB) are bacteria that can facilitate organic matter degradation and nutrient release as well as conducting photosynthesis [9]. Their metabolic activities are also believed to be linked to the stabilization of soil organic carbon [10]. Past research has shown that PNSB can improve the root zone ecosystem and stimulate plant growth hormone synthesis, providing dual positive effects on soil health and crop growth [11]. For these reasons, they exhibit considerable potential for sustainable agricultural development.

This study consists of systematic optimization experiments based on the Taguchi Method. It investigates four management factors: amount of fertilizer, the amounts of two different photosynthetic bacteria added as supplements, and irrigation water volume. Response analyses were conducted on crop indicators such as chlorophyll content, plant height, fresh weight, and dry weight, exploring the comprehensive impacts of different management technique combinations on corn growth and soil carbon sequestration. The study aims to provide practical management recommendations to further enhance carbon sequestration potential and achieve sustainable agricultural goals [12].

II. MATERIALSAND METHODS

A. *ExperimentalDesign*

This study was conducted in a greenhouse using pot experiments to simulate field cultivation conditions and systematically evaluate the effects of different factors on corn growth and soil carbon sequestration. The experiment was designed according to the Taguchi Method, which established an L9 (3^4) orthogonal array that included four control factors, with each factor set at three levels, combining to form nine treatment groups. The duration of the experiment was three months.

Control factors included: fertilization amount, the amount of PNSB *Rhodopseudomonas* sp. strain AKW01 added, the amount of PNSB *Rhodoplanes* sp. strain AKW02 added, as well as the volume of daily irrigation water, simulating different nutrient and water management conditions. The bacterial solutions were provided in a liquid form, and it was periodically diluted and injected into the soil throughout the experiment starting from the earliest stage of the experiment, to promote the colonization and proper function of the PNSB [13].

Fertilization management was adjusted based on local corn cultivation and experimental recommendations, with three fertilization levels set at 100, 130, and 160 kg/ha. The application rates of the bacterial solution were set at 0, 5, and 10 mL/plant; irrigation water volume was set at 600, 800, and 1000 mL/day, which simulated low, medium, and high water supply conditions.

During the experiment, each plant was evaluated at each of the various growth stages of corn. Growth indicators that were recorded included chlorophyll content as determined using a Soil Plant Analysis Development (SPAD) meter, plant height, and fresh and dry weights which were measured at the final harvest. Soil samples were also collected, and physicochemical properties such as pH, electrical conductivity (EC), and soil organic carbon (SOC), were analyzed. These measurements served as response variables for subsequent Taguchi analysis and factor effect evaluations.

B. Control Factor LevelSetting

To optimize the analysis of corn growth and soil carbon sequestration potential, the experiment selected four control factors: fertilization amount, AKW01 strain addition ratio, AKW02 strain addition ratio, and irrigation water volume, as the main variables for the experimental design. The selection of control factors was based on observations from preliminary experiments, corn cultivation experience, and relevant literature on microbial applications, balancing practical operation with research representativeness, aiming to effectively explore the overall benefits of nutrient and water management on crop growth and carbon fixation potential.

The three level settings for each control factor were aimed to simulate possible management ranges in practice. Fertilization levels were chosen based on recommended dosages for corn cultivation in Taiwan; amounts of AKW01 and AKW02 PNSB addition amounts were set based on stability as determined in prior experiments; and irrigation water volume was set to simulate different water supply conditions commonly seen in irrigation management [14] .

The level settings for each control factor are shown in Table 1, and serves as the basis for the subsequent L9 (3^4) orthogonal array configuration, as well as the ensuing Response Analysis and Factor Effect evaluations.

TABLE I

LEVEL SETTINGS FOR DIFFERENT CONTROL FACTORS

Control factor	Level 1	Level 2	Level 3
Fertilization amount	100 kg/ha	130 kg/ha	160 kg/ha
Amount of strain AKW01	0 ml/plant	5 ml/plant	10 ml/plant
Amount of strain AKW02	0 ml/plant	5 ml/plant	10 ml/plant
Irrigation volume	600 ml/day	800 ml/day	1000 ml/day

C. L9 Orthogonal Array Configuration

To optimize the levels of the control factors in this study, this study adopted the Taguchi Method and selected an experimental configuration featuring a L9 (3^4) orthogonal array. This design allows for the effective exploration of multi-factor and multi-level combinations within a limited number of experiments, enhancing overall experimental efficiency and analytical resolution. The L9 Orthogonal Array accommodates four control factors, each with three levels. Through a systematic arrangement of factors and levels, a total of nine treatment groups are formed, which are representative of possible real world conditions, and enables statistical comparison between groups [15].

Based on the control factors and level settings shown in Table 1, the factors were combined according to the orthogonal array arrangement to establish the L9 experimental configuration used in this study, as shown in Table 2. This combination will serve as the basis for the actual pot experiments that were conducted, and then followed by measurements of the response variables and statistical analyses

TABLE IIIII
LEVELS OF EACH CONTROL FACTOR IN THE L9 ORTHOGONAL ARRAY

Number	Fertilization amount (kg/ha)	Amount of strain AKW01 (ml/plant)	Amount of strain AKW02 (ml/plant)	Irrigation volume (ml/day)
L1	100	0	0	600
L2	100	5	5	800
L3	100	10	10	1000
L4	130	0	5	1000
L5	130	5	10	600
L6	130	10	0	800
L7	160	0	10	800
L8	160	5	0	1000
L9	160	10	5	600

D. *Response Variables*

The experiment selected three crop growth indicators — chlorophyll content, fresh weight, and dry weight of the corn plants — as response variables, to assess the impact of different management factors on crop performance. Chlorophyll content serves as a critical indicator of plant physiological activity by indicating the photosynthetic efficiency and nutritional status of the plants; fresh weight represents the accumulation of biomass and reflects the overall growth potential and water usage of the crop; and dry weight is used as an indicator of the final biomass of the plants and is also key for assessing carbon fixation capacity and yield potential [16]. All three Response Variables were measured at the corn harvest stage, and the data obtained were used to calculate the Signal-to-Noise Ratio (SNR) according to the Taguchi method. Factor effect analysis was also used to determine the relative contributions of each management factor and the optimal combination, thereby providing a basis for subsequent agricultural management strategy adjustments and sustainable cultivation recommendations [17].

$$\mathrm{SNR} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_{i^2}}\right)$$

with n *being the number of levels for each variable*, $n = 3$

E. *Data Analysis*

This study employed the Taguchi method for data analysis and calculated the SNR for each treatment combination under different response variables based on the “larger-the-better” objective function. This method enables the assessment of the stability of each combination’s performance, as well as enhances data resolution and discernibility. The calculated SNR values for the three Response variables (chlorophyll, fresh weight, and dry weight) served as the basis for subsequent factor effect analysis.

Next, the average SNR values for each factor at different levels were compiled, and factor effect diagrams were created to observe the influence of each control factor on the response variables, further elucidating the optimal parameter combinations.

Additionally, we used analysis of variance (ANOVA) analyses to quantify the contributions of each control factor to the total variation. We calculated the sum of squares (SS) for each individual factor and deduced the correction factors (CF) to find the percentage contribution of each factor. Significant control factors were systematically identified, and the corresponding optimal level combinations could serve as the foundation for subsequent strategic planning to enhance the carbon sequestration potential of corn.

$$SS_{\mathrm{A}} = r \cdot [(\bar{y}_{A1} - \bar{y})^2 + (\bar{y}_{A2} - \bar{y})^2 + (\bar{y}_{A3} - \bar{y})^2]$$

r：number of levels of each variable, r=3

$\bar{y}_i$：average SNR of the i^{th} level

$\bar{y}$：overall average SNR

$$CF = \frac{(\sum \mathrm{y})^2}{n}$$

$\sum$y=summation of SNR in all experimental groups

n=total number of groups, n=9

$$Percentage\ contribution = (\frac{SS_{\mathrm{Factor}}}{SS_{\mathrm{total}}}$$

SS_{factor}：sum of squares of a specific factor

SS_{total}：sum of squares of all factors

F. *Organic Carbon*

Based on the soil organic matter determination method published by the Agricultural Research Institute of the Council of Agriculture in Taiwan, TARI S201.1B, we applied the combustion/infrared measurement method to soil samples, which were pre-treated and then analyzed with a total organic carbon (TOC) analyzer, ensuring the accuracy and reproducibility of the data [18].

III. RESULTS AND DISCUSSION

A. *Factor Effect Diagrams and Optimal Combinations for Individual Response Variables*

1) *Chlorophyll*

The SNR value for chlorophyll was highest for fertilization amount, indicating that said variable had a significant impact on chlorophyll content. Water volume ranked next in importance. Based on an analysis of all factors, the estimated optimal parameter combination for chlorophyll performance is level 3 for fertilization amount, level 3 for AKW01 strain amount, level 2 for AKW02 strain amount, and level 1 for water volume, as shown in Figure 1. This finding underscores the critical role of fertilization, particularly nitrogen input, as the dominant factor driving chlorophyll accumulation[19].

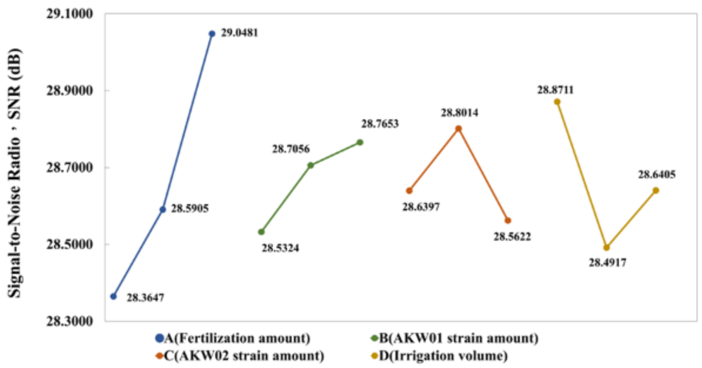

Fig. 1Effects of different factor levels on the chlorophyll SNR in pot experiments.

2) Fresh Weight

The addition of the AKW01 strain PNSB and irrigation water volume were the primary factors affecting SNR for fresh weight. Fertilization amount was a secondary factor, while the amount of AKW02 strain added had an even lower impact. The overall results predict that the optimal combination for fresh weight is level 2 for fertilization amount, level 3 for AKW01 strain amount, level 1 for AKW02 strain amount, and level 1 for water volume, as depicted in Figure 2. This result highlights the dominant role of AKW01 application in promoting biomass accumulation[20].

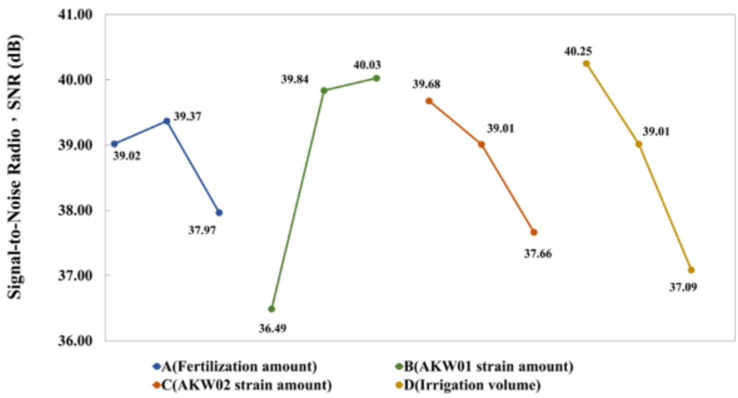

Fig. 2 Effects of different factor levels on the fresh weight SNR in pot experiments.

3) Dry Weight

The variability in SNR for dry weight was primarily influenced by the addition of the AKW01 strain and the fertilization amount, followed by water. The estimated best combination of factor for optimizing dry weight is level 2 for fertilization amount, level 3 for AKW01 strain amount, level 2 for AKW02 strain amount, and level 1 for water volume, as shown in Figure 3. This finding confirms the predominant influence of AKW01 supplementation on enhancing final biomass accumulation[20].

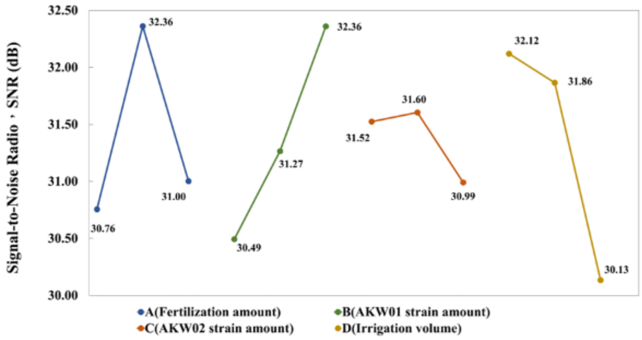

Fig. 3 Effects of different factor levels on the dry weight SNR in pot experiments.

There are slight differences in the predicted optimal combinations for the three response variables; however, the optional amounts of AKW01 strain addition and irrigation water volume were consistent across levels, indicating stable positive effects on corn growth. The predicted optimal combinations for each response variable are summarized in Table 3.

TABLE IVVVI
PREDICTED OPTIMAL COMBINATIONS FOR EACH RESPONSE VARIABLE IN POT EXPERIMENTS.

Variable	Fertilization amount	AKW01 strain amount	AKW02 strain amount	Irrigation volume
Chlorophyll	Level 3	Level 3	Level 2	Level 1
Fresh weight	Level 2	Level 3	Level 1	Level 1
Dry weight	Level 2	Level 3	Level 2	Level 1

B. *ANOVA Results*

To further understand the influence of each control factor on different response variables, this study conducted an analysis of variance (ANOVA) based on the SNR signal-to-noise analysis results to calculate the contribution percentage of each factor to total variation. This allows for an assessment of the degree of influence and importance of each factor on the overall outcome. The compiled data is presented in Table 4 [21].

1) Chlorophyll

The ANOVA analysis revealed that the main contributing factor for chlorophyll was the fertilization amount, accounting for 64.72% of total contribution, indicating a high level of influence on chlorophyll content. The other contributing factors in descending order of significance were: AKW01 strain application (19.51%), water volume (7.96%), and AKW02 strain application (7.81%). These results reflect the close relationship between crop photosynthesis, nitrogen accumulation, and fertilization strategy, which is consistent with previous studies showing that nitrogen fertilizer significantly enhances leaf chlorophyll concentration and photosynthetic efficiency [21,22].

2) Fresh Weight

The AKW01 strain was the most significant factor affecting fresh weight, contributing to 48.94% of the variance, indicating its strong role in promoting plant biomass accumulation. Irrigation volume was the second most influential factor at 31.47%, followed by the AKW02 strain at 13.00%, and fertilization amount at 6.59%. These findings suggest that microbial inoculants, particularly the AKW01 strain, have a greater impact on fresh biomass than irrigation under the tested conditions [23].

3) Fresh Weight

The factor with the highest contribution to dry weight was irrigation volume (40.16%), followed by the AKW01 strain amount (30.27%) and fertilization amount (25.74%), while the AKW02 strain contributed only 3.82%. These results indicate that irrigation played the most decisive role in dry biomass accumulation, likely due to its influence on water availability and transpiration efficiency [24]. The significant contribution of the AKW01 strain suggests its potential role in enhancing nutrient uptake and promoting plant growth[23].

TABLE VIIV

CONTRIBUTIONS OF DIFFERENT FACTORS TO VARIOUS PLANT INDICATORS IN POT EXPERIMENTS.

Factor	Fertilization amount	AKW01 strain amount	AKW02 strain amount	Irrigation volume
	------(%)------			
Chlorophyll	64.72	7.81	7.96	19.51
Fresh weight	6.59	48.94	13.00	31.47
Dry weight	25.74	30.27	3.82	40.16

Overall, the AKW01 strain of PNSB consistently contributed positively to all three response variables, while the impact of water and fertilization varied by response variable. The contribution of the AKW02 strain ratio was generally low, indicating limited effects of said strain on corn growth. Further evaluation of its interactions with other crops may be warranted. Although the main factors influencing each individual indicator varied, the integrated analysis showed that the AKW01 strain and irrigation volume were the most influential overall. This highlights the importance of using a comprehensive approach when optimizing multiple aspects of crop performance.

C. Overall Benefits of Response Factors

To integrate the results of crop response variables and elucidate the optimal management combination, this study employed multi-response signal-to-noise (MRSN) analysis to normalize and weigh the three response variables, and calculate the comprehensive SNR for each treatment combination. Factor effect diagrams were plotted to observe the contributions of factors to overall benefits, as illustrated in Figure 4 [26].

The results showed that the AKW01 strain and water volume exhibited the greatest variability across different levels, thereby significantly influencing overall crop performance. The predicted optimal combination is level 2 for fertilization amount, level 3 for AKW01 strain amount, level 2 for AKW02 strain amount, and level 1 for water volume.

$$SNR_i^{**} = w_1 \times SNR^*_{i_{Chlorophyll}} + w_2 \times SNR^*_{i_{FreshWeight}}$$

Weighting values assignment
Chlorophyll (w1):0.2
Fresh weight (w2):0.3
Dry weight (w3):0.5
w1+ w2+ w3= 1

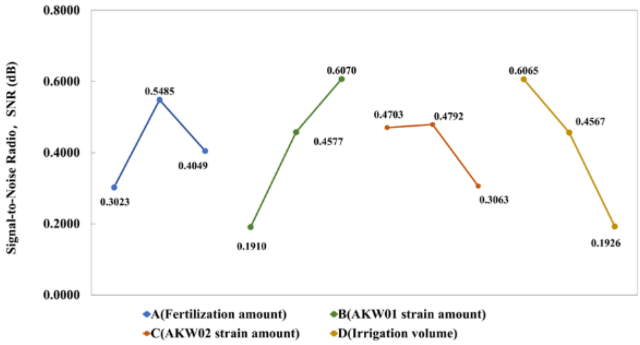

Fig. 4 Overall benefit effects of various factors at different levels based on MRSN in pot experiments.

An additional analysis using the root mean square (RMS) integration model suggested slightly different optimal combinations, which were level 2 for fertilization amount, level 3 for AKW01 strain amount, level 1 for AKW02 strain amount, and level 1 for water volume. Both calculation models highlighted the stable advantages of level 3 for the AKW01 strain amount and level 1 for water volume, indicating their key roles in promoting crop performance across multiple objectives. Furthermore, the convergence of results across distinct integration approaches underscores the robustness of these factors, suggesting that the consistent benefits derived from AKW01 supplementation and controlled irrigation are not artifacts of a specific analytical method but rather reflect fundamental biological and agronomic mechanisms.

$$SNR_i^{***} = \sqrt{\left(w_1 \times SNR^*_{i_{Chlorophyll}}\right)^2 + \left(w_2 \times SNR^*_{i_{FreshWeight}}\right)^2 + \left(w_3 \times SNR^*_{i_{DryWeight}}\right)^2}$$

Weighting values assignment
Chlorophyll (w1):0.2
Fresh weight (w2):0.3
Dry weight (w3):0.5
w1+ w2+ w3= 1

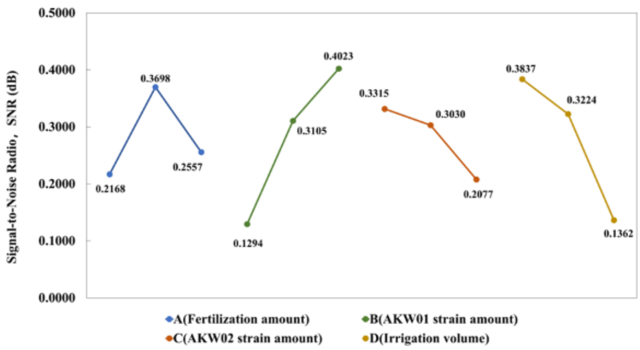

Fig. 5 Overall benefit effects of various factors at different levels based on RMS in pot experiments.

In conclusion, the application of the AKW01 strain consistently led to stable contributions in crop performance across all analysis results. Additionally, crops performed best at lower irrigation volume levels. The impacts of fertilization amount and AKW02 strain addition varied slightly depending on the integration model. The contribution percentages of each factor at different levels to plant growth indicators are summarized in Table 5, offering detailed insight into their respective influences. The predicted optimal combinations corresponding to each response variable and the integrated models are presented in Table 6. Taken together, these findings indicate that the convergent outcomes of multiple analytical approaches strengthen the reliability of AKW01 supplementation and irrigation control as robust strategies for optimizing both crop yield and soil carbon sequestration.

TABLE V
Contributions of different factors to various plant indicators in pot experiments.

Factor	Fertilization amount	AKW01 strain amount	AKW02 strain amount	Irrigation volume
MRSM	13.52%	39.27%	8.38%	38.83%
RMS	13.62%	41.55%	9.05%	35.78%

TABLE VI
CONTRIBUTIONS OF DIFFERENT FACTORS TO VARIOUS PLANT INDICATORS IN POT EXPERIMENTS.

Factor	Fertilization amount	AKW01 strain amount	AKW02 strain amount	Irrigation volume
MRSM	Level 2	Level 3	Level 2	Level 1
RMS	Level 2	Level 3	Level 2	Level 1

D. *Soil Organic Carbon*

After completing the plant growth experiments, this study also analyzed the effects of each factor on soil organic carbon (SOC) SNR. The results, shown in Figure 6, indicate that the fertilization amount at Level 3 (160 kg/ha) had a significant positive impact on SOC, suggesting that adequate fertilization aids carbon accumulation. This aligns with global findings showing that nitrogen fertilization significantly increases particulate and mineral-associated organic carbon, especially in carbon-depleted soils [27]. The AKW01 strain amount at Level 3 (10 ml/plant) was also associated with the highest SNR, indicating that adding higher amounts of the PNSB strain to soil can enhance carbon fixation, which supports evidence that microbial inoculants improve soil carbon stability and structure [28]. The AKW02 strain at Level 2 (5 ml/plant) was found to be more advantageous for SOC stability, possibly linked to microbial diversity enhancing the stabilization of mineral-associated organic carbon under variable environmental conditions [29]. Meanwhile, water volume showed a slight decreasing trend in SNR with increased levels, indicating that excessive irrigation might lead to carbon loss, consistent with reports that irrigation volume is negatively correlated with SOC retention in global croplands [30]. Overall, these results indicate that SOC stability is primarily influenced by the management of microbial strain application and fertilization strategies.

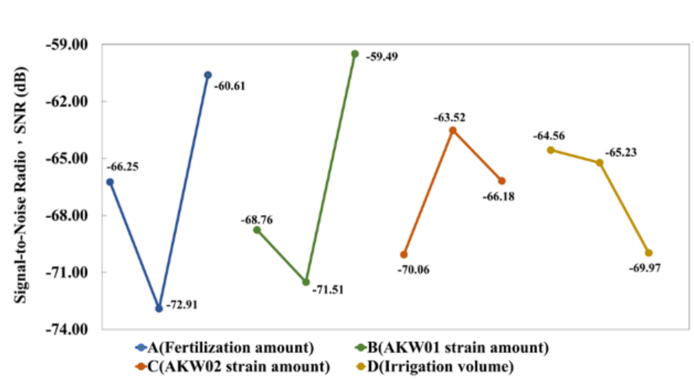

Fig. 6 Effects of various factors on soil organic carbon SNR in pot experiments.

IV. CONCLUSIONS

This study utilized the Taguchi method of experimental design to systematically assess the impacts of four crop management factors — fertilization amount, addition of purple non-sulfur bacteria strains AKW01 and AKW02, and irrigation water volume — on corn growth and soil carbon sequestration potential. Multi-response signal-to-noise ratio (SNR) and analysis of variance (ANOVA) tests were used to evaluate the effects of these factors, and an integrated analysis was used to elucidate the optimal combination of factors.

The results of this study indicate that the addition of PNSB strain AKW01 was the most prominent contributing factor that improved plant indicators such as chlorophyll content, fresh weight, and dry weight, while also effectively enhancing soil organic carbon (SOC) content. This highlights the significance of AKW01 strain application as a key technology for achieving the dual goals of promoting carbon fixation and increasing crop yield. Irrigation water volume was also a significant factor, that in particular impacted the fresh weight and dry weight of plants. This demonstrates the potential of regulating biomass accumulation using water management strategies.

Overall, we can infer from the results of the individual and integrated analyses that addition of the AKW01 strain and management of the water supply should be prioritized in planning sustainable corn cultivation. The individual contribution of the AKW02 strain was relatively low, suggesting that its interactions with other microbes and crops could be explored in the future.

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