Evaluation of organic substitution based on vegetable yield and soil fertility

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ABSTRACT
Recultivation of abandoned farmland (AF) is a supplement to land resources. However, less is known about the effects of different organic substitution rates (OSRs) on soil fertility under AF recultivation. We investigated the response of vegetable yields and soil physicochemical and microbial properties to five OSRs (0, 25%, 50%, 75%, and 100%) in an AF. Single chemical fertilizer treatment (0 OSR) had the highest yield (54.4 and 24.8 t hm⁻²) and nitrate content (1118.8 and 341.5 mg kg⁻¹) of Chinese cabbage and spinach. With the increase of OSRs, vitamin C content gradually increased in both vegetables. Organic substitution had a positive effect on the main soil fertility indicators. Although 50% and 75% OSRs had not the highest vegetable yield, they significantly improved vegetable quality and soil fertility. Therefore, it is feasible to increase or maintain soil fertility by organic substitution under AF recultivation.

Soil fertility is a key factor affecting crop yield, quality and production sustainability, including physical, chemical and biological fertility [1]. Soil microbial properties, such as microbial biomass and enzyme activities, are the main indicators of soil biological fertility [2]. The size of the microbial biomass and enzyme activity can directly reflect the level of soil fertility [3]. Because soil microbial indicators are sensitive to changes in soil environment, they are often considered as early warning indicators of soil quality changes [4,5].

In recent years, farmland abandonment has attracted much attention due to its close relationship with the shortage of cultivated land resources and food security, and the recultivation of idle and abandoned farmland has risen to the national level in China [6,7]. Since all plant residues are returned to the soil under abandoned conditions, long-term abandoned soil often has a high fertility [8,9]. However, some studies have found that although the aggregate quality, organic carbon (SOC) content, enzyme activity and microbial biomass in the soil of long-term abandoned land is high, their stability is poor [10]. In order to avoid the reduction of soil fertility when abandoned land is reused, it is therefore necessary to pay more attention to the maintenance of soil fertility for its recultivation.

Fertilization is one of the most obvious agricultural measures to influence soil fertility [11,12], but excessive application of chemical fertilizer will lead to soil acidification, imbalance of soil nutrient proportion and decline of agricultural sustainable production capacity [13,14]; partial substitution of chemical fertilizers by organic amendments is considered to be an effective measure to reduce chemical fertilizer input and ensure sustainable soil utilization [15]. In this context, it is necessary to study the effects of different organic fertilizer substitution rates (OSRs) on soil microbial biomass and enzyme activity. Some studies found that the combined application of organic and inorganic fertilizer can significantly increase soil microbial biomass and enzyme activity, which is an effective measure to improve soil fertility [16–18]. However, there is still a lack of discussion on the response of soil physicochemical and microbial properties to different OSRs in idle and abandoned agricultural land.

To investigate the effect of organic fertilizer substitution on vegetable yield and soil fertility in a soil experienced long-term abandonment, a field trial with different OSRs applied to Chinese cabbage and spinach was set up in a long-term (25 years) abandonment farmland. The hypothesis of this study was that, under the recultivation of long-term abandonment agricultural soil, partial substitution of chemical fertilizer by organic fertilizer is essential for improving or maintaining the soil fertility without unacceptable yield loss compared to the single application of chemical fertilizer. Thus, six treatments were conducted, including no fertilizer treatment and five OSR treatments (0, 25%, 50%, 75% and 100% of chemical N fertilizer substituted with organic fertilizer). The objectives of this study were to investigate the response of physicochemical and microbial properties to different OSRs and the optimal OSR for the vegetable production and soil

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fertility in abandoned farmland. This study can provide a scientific basis for better maintaining soil fertility of abandoned farmland and rational application of organic fertilizer.

1 Materials and methods

1.1 Field site and experiment design

The study area is located in an experimental field of Shandong Agricultural University, Tai’an, Shandong Province, China (117°1’ E, 36°1’ N). The mean annual temperature and the mean annual precipitation are 12.9°C and 650 mm, respectively. The soil is silty loam (USDA system) mainly developed from diluvial deposits. The experimental field has been abandoned since 1992. Prior to the organic fertilizer substitution experiment, the soil (0–20 cm) properties were bulk density 1.21 g cm⁻³, SOC 9.56 g kg⁻¹, total N (TN) 0.85 g kg⁻¹ and pH (H₂O) 7.30.

The application rates of N, P₂O₅, and K₂O for all fertilization treatments in this study were 165, 150, and 150 kg ha⁻¹, respectively. Organic fertilizer substitution was calculated according to N rate. The applied organic fertilizer was pig manure, which was produced by the local organic fertilizer factory. The organic fertilizer contains 42% of organic matter, 2.2% of N, 1.4% of P₂O₅, 1.6% of K₂O. Urea (N 46%), diammonium phosphate (N 14%, P₂O₅ 44%) and potassium sulfate (K₂O 50%) were used as chemical fertilizer.

Chinese cabbage and spinach were cultivated under field experiment in 2017.09–2018.05. Six treatments were conducted in a randomized block design with four replications (each plot was 3 m × 3 m = 9 m²), including no fertilizer (denoted as CK) and 0, 25%, 50%, 75%, and 100% of chemical N fertilizer substituted with organic fertilizer (denoted as CF, M25, M50, M75, and M100, respectively). Details of nutrient application rates are presented in Table 1. The flowchart of this study is shown in Figure 1.

Chinese cabbage was sown on 4 September 2017. Sixty percent of the fertilizers were mixed thoroughly with 0–20 cm soil as basal fertilization and others were applied as topdressing. The seedlings were fixed at the end of the seedling stage (23 September). The yield was determined on 3 December 2017. For spinach, all the fertilizers were ploughed into the soil in the form of base fertilizer before sowing. The spinach was harvested on 17 May 2018.

![Figure 1. The flowchart of this study.](image-url)
1.2 Soil sampling and analysis

Soil samples were collected on 17 May 2018. Three soil cores (0–20 cm) were randomly selected from each plot and mixed after removing debris. One part of the samples was stored at 4°C and the SMBC, SMBN and SMBP were determined immediately. The other part of the samples was air-dried to measure soil pH, SOC, TN, available P (AP), available K (AK), and the activities of urease, catalase, sucrose, and phosphatase. Undisturbed soil in natural state was collected from each plot using a 100 cm³ ring-knife to determine soil bulk density and soil porosity (SP). Meanwhile, three undisturbed soil clods (20 cm × 20 cm × 10 cm) were also sampled in each plot to analyze soil aggregates and determine mean weight diameter (MWD).

SOC and TN contents were determined by an elemental analyzer (Model CN, vario Macro Elementar, Germany). Soil pH was measured using a glass electrode in a 1:2.5 soil-water suspension. Soil AP was determined using molybdenum blue method after extraction by 0.5 M NaHCO₃ [19]. Soil AK was extracted by 1 M NH₄OAc (pH 7.0) and determined by flame photometry (FP640, INASA, China).

SMBC, SMBN and SMBP were extracted by chloroform fumigation [17]. SMBC was determined by potassium sulfate extraction and K₂Cr₂O₇ oxidation method, and the conversion coefficient was 0.45; SMBN was determined by potassium sulfate extraction ninhydrin colorimetry, and the conversion coefficient was 0.45; SMBP was determined by NaHCO₃ extraction and molybdenum blue colorimetry, and the conversion coefficient was 0.40. The calculation equations of SMBC, SMBN, and SMBP are as follows [20]:

\[
\text{SMBC (mg kg}^{-1}\text{)} = (\text{fumigated C-non fumigated C})/0.45 \text{ (1)}
\]

\[
\text{SMBN (mg kg}^{-1}\text{)} = (\text{fumigated N-non fumigated N})/0.45 \text{ (2)}
\]

\[
\text{SMBP (mg kg}^{-1}\text{)} = (\text{fumigated P-non fumigated P})/0.40 \text{ (3)}
\]

Soil catalase activity, sucrase activity, urease activity, and neutral phosphatase activity were determined by potassium permanganate titration, 3, 5-Dinitrosalicylic acid colorimetry, sodium phenol sodium hypochlorite colorimetry, and disodium phenylphosphate colorimetry, respectively [21].

1.3 Statistical analysis

Statistical analysis was carried out with the SPSS 16.0 package (SPSS, Chicago, Illinois, USA). Normality and homogeneity of variance of data were tested prior to analyses, and the data were ln-transformed if necessary. One-way Analysis of Variance (ANOVA) was performed to analyze the effects of OSRs on the yield and quality of vegetable crops, and soil fertility. Tukey’s tests were used to examine the differences in the mean values between treatments. A level of 0.05 was chosen to indicate statistical significance. Comprehensive evaluation of soil fertility under different OSRs were compared and analyzed using principal component analysis (PCA). PCA was conducted with the standard setting in the program CANOCO 4.5.

2 Results

2.1 Effect of different OSRs on the yield and quality of Chinese cabbage and spinach

In the present study, fertilizer application significantly increased the yields of Chinese cabbage and spinach compared with CK treatment (Figure 2). For Chinese cabbage and spinach, the greatest yields were all observed in CF treatment, which significantly increased yields by 12.66–43.94% compared with the other five treatments (P < 0.05). With the increase of OSRs, the Chinese cabbage and spinach yields decreased and M100 treatment had the lowest yields for both vegetables (Figure 2).

Nitrate content is an important indicator of vegetable quality. Compared with CK treatment, fertilization significantly increased the nitrate content of Chinese cabbage and spinach (P < 0.05, Table 2). CF treatment had the highest nitrate content among the fertilization treatments regardless of vegetable type. With the increase of OSRs, the nitrate content of vegetables decreased.

Vitamin C (VC) is an indispensable nutrient for human life activities, mainly from vegetables and other food intake. The total amount of VC is one of the important indexes to evaluate the quality of vegetables. Compared with CK treatment, the single application of chemical fertilizer (CF treatment) reduced the total amount of VC in Chinese cabbage and spinach by 26.55% and 38.49%, respectively (P < 0.05). However, with the increase of OSRs, the total amount of VC significantly increased and M100 treatment had the largest total amount of VC in both vegetables (Table 2).

2.2 Effect of different OSRs on soil physicochemical properties

CF treatment showed lower SP and MWD compared with CK treatment. With the increase of OSRs, the SP and MWD had a significant increase (P < 0.05). Compared with CK treatment, fertilization decreased soil pH and C/N, and the differences between CF or M25 treatment and CK treatment were significant (P < 0.05). The reduction of soil pH and C/N by CF treatment was higher than that by M25 treatment. The contents of SOC and TN in M75 treatment were the highest among all treatments: compared with CF and M25 treatment, M75 treatment increased SOC content by 20.86% and 14.44%, respectively; the
content of TN was increased by 11.00% relative to CK treatment. Compared with CK treatment, CF, M25, M50, M75 and M100 treatment increased AP content by 54.58%, 54.97%, 48.24%, 43.23% and 39.29%, respectively (Table 3). CF treatment had the highest AK content among the six treatments, while M100 treatment had the lowest AK content.

### 2.3 Effect of different OSRs on soil microbial properties

Fertilization significantly increased SMBC, SMBN, and SMBP content compared with CK treatment (P < 0.05). Among the five fertilization treatments, M75 treatment had the highest SMBC and SMBN content (Figure 3(c)). The ratio of SMBC to SOC (SMBC/SOC) is named as microbial quotient (QMB), which can be used to indicate the microbial availability of soil organic matter and can directly or indirectly reflect the changes of soil quality in different restoration stages. Fertilization significantly increased the value of QMB (P < 0.05), and M75 treatment had the highest QMB value (Figure 3(d)).

Fertilization treatments reduced soil catalase activity by 0.68–20.73%, but with the increase of OSRs, the reduction of catalase activity decreased significantly (Figure 4(a)). The differences in soil sucrase activity between fertilization treatments and CK were not significant (P > 0.05) (Figure 4(b)). Soil urease activity ranged from 0.99 to 1.32 (mg NH₃-N g⁻¹ soil, 24 h). CF treatment had the lowest urease activity, while M75

![Figure 2](image.png)  
Figure 2. Chinese cabbage and spinach yields (fresh weight) under different organic substitution rates. Error bars mean standard deviations (n = 3). Different letters above the bars indicate significant differences (p < 0.05) in Chinese cabbage or spinach yields among treatments.

### Table 2. Effect of organic substitution rates on quality of Chinese cabbage and spinach.

| Treatment | Chinese cabbage | Spinach |
|-----------|----------------|---------|
|           | Nitrate (mg kg⁻¹) | Vitamin C (mg kg⁻¹) | Nitrate (mg kg⁻¹) | Vitamin C (mg kg⁻¹) |
| CK        | 805.97 ± 26.23e | 219.49 ± 12.92ab | 248.76 ± 20.77e | 801.43 ± 16.30bc |
| CF        | 1118.80 ± 73.04a | 172.27 ± 18.95c | 341.54 ± 18.94a | 730.88 ± 13.27d |
| M25       | 981.53 ± 36.59b | 211.72 ± 13.02b | 330.99 ± 15.92ab | 787.00 ± 29.00c |
| M50       | 941.98 ± 13.44bc | 218.83 ± 7.68ab | 308.10 ± 17.24bc | 804.11 ± 7.53bc |
| M75       | 909.42 ± 15.20 cd | 226.27 ± 14.24ab | 289.54 ± 13.02 cd | 828.21 ± 15.96ab |
| M100      | 847.53 ± 34.75de | 240.85 ± 10.74a | 277.45 ± 9.33de | 846.13 ± 10.40a |

Note: Different letters in the same column indicate the significant difference at 5% level.

### Table 3. Soil physicochemical properties under different fertilizer treatments.

| Treatment | SP (%) | MWD (mm) | pH (H₂O) (2.5:1) | SOC (g kg⁻¹) | TN (g kg⁻¹) | C/N (SOC/TN) | AP (mg kg⁻¹) | AK (mg kg⁻¹) |
|-----------|--------|----------|------------------|-------------|-------------|--------------|--------------|--------------|
| CK        | 51.82de| 0.78bc   | 7.31a            | 9.27bc      | 0.81b       | 11.52a       | 62.94b       | 75.48bc      |
| CF        | 50.44d | 0.76 cd  | 6.42c            | 8.46c       | 0.85ab      | 10.01c       | 97.29a       | 96.10a       |
| M25       | 51.57cd| 0.79bc   | 6.80bc           | 8.93bc      | 0.87ab      | 10.21bc      | 97.54a       | 86.16abc     |
| M50       | 52.95bc| 0.81abc  | 6.98ab           | 9.66ab      | 0.91a       | 10.65abc     | 93.30a       | 89.52ab      |
| M75       | 53.71b | 0.82ab   | 7.04ab           | 10.22a      | 0.92a       | 11.07ab      | 90.15a       | 90.55ab      |
| M100      | 55.35a | 0.85a    | 7.14ab           | 9.62ab      | 0.86ab      | 11.23ab      | 87.67a       | 72.79c       |

Note: SP, soil porosity; MWD, mean weight diameter; SOC, soil organic carbon; TN, total nitrogen; C/N, SOC/TN; AP, available phosphorus; AK, available potassium. Different letters in the same column indicate the significant difference at 5% level.
Comprehensive evaluation of soil fertility under different OSRs

The results of PCA showed that soil microbial and physicochemical properties were markedly affected by fertilization and OSRs. PC1 accounted for 39.48% and PC2 accounted for 31.61% of the variation in microbial and physicochemical properties (Figure 5). PC1 axis differentiated the samples treated with higher OSRs from samples treated with CK, chemical fertilizer, and lower OSRs (M25), whereas PC2 axis differentiated the samples with fertilizer application (CF, M25, M50, and M75) from samples without fertilizer application (CK) and 100% OSR (M100).

The M50 and M75 treatment with higher PC1 and PC2 scores were observed on the right side of the PC1 axis and on the upper side of the PC2 axis.

3 Discussion

It was known that the application of chemical fertilizer can effectively improve crop yield and soil nutrient content, but the effect on improving soil microbial environment and sustainable utilization is not as good as that of organic fertilizer [22,23]. In this study, we found that the application of chemical fertilizer significantly increased the yield of Chinese cabbage and spinach and decreased the quality (indicated by the nitrate and VC content) (Figure 2, Table 2). With the increase of OSRs, the yield of Chinese cabbage and spinach decreased, while the quality has been improved to a certain extent. These results showed that the reasonable ratio of organic fertilizer and inorganic fertilizer can increase vegetable yield and quality, and large amount of chemical fertilizer input may lead to vegetable quality reduction. However, some studies have reported that the combined application of organic and inorganic fertilizers plays a much more
important role in increasing the yield of crops compared with a single application of chemical fertilizer [24,25]. This is inconsistent with our results. We present possible reasons for this phenomenon: (1) the soil of our research was long-term abandoned agricultural soil without available nutrients application and most of the nutrients in organic fertilizer were in slow-released state, thus vegetable yield was mainly limited by the application rate of available nutrients; (2) the yield response is related to the length of planting time. Yang et al. [26] also found that the yield increase effect of single application of chemical fertilizer is better than that of high-volume organic fertilizer combined with inorganic fertilizer, but with the extension of time, the yield increase of high-volume organic fertilizer combined with chemical fertilizer is greater.

Long-term single application of chemical fertilizer may lead to negative effects on soil physicochemical properties, while organic and inorganic applications are an effective way to improve or maintain soil physicochemical properties [27,28]. Our results also showed that organic substitution improved SP and MWD compared with the single application of chemical fertilizer. Fertilization decreased soil pH value, and the effect of CF treatment was the most obvious, while organic substitution can effectively alleviate the lowering effect (Table 3), which is consistent with previous views that organic fertilizer application has buffering effect on soil pH value [29]. Fertilization can significantly improve the AP content of soil, and CF treatment was the highest, which is related to its greatest phosphorus application amount. Our study also showed that the SOC and TN were the highest under 75% OSR (M75), and the effect of this OSR on maintaining soil chemical fertility was better than other OSRs.

Soil enzyme activity is used as an early warning biological indicator of soil change because of its sensitivity to the environment change, especially agricultural soil change caused by pollution, management measures, planting system, land use, etc. [30,31]. Generally, the long-term abandoned soil had higher activities of soil catalase, sucrase and urease. But a single application of chemical fertilizer decreased these three enzyme activities. This result indicated that under the recultivation of long-term abandonment agricultural soil, the single application of chemical fertilizer may reduce the soil microbial fertility and affect the soil health and element cycle. Some previous studies found that the activities of soil sucrase, urease and neutral phosphatase in organic fertilizer treatment were significantly higher than those in control and single N fertilizer treatment, and soil enzyme activity in organic fertilizer combined with chemical N fertilizer treatment was higher than that in single organic fertilizer treatment [32]. Our results are consistent with the
results of Sun et al. [33] and Liu et al. [34], who found that fertilization reduced some soil enzyme activity, and the greater the proportion of chemical fertilizer, the greater the reduction. Although organic fertilizer substitution did not significantly improve enzyme activity compared to the CK treatment, it played an important role in maintaining enzyme activity of the long-term abandoned soil [35].

Soil microbial biomass can reflect the assimilation and mineralization capacity of soil organic nutrients [2]. The results of some previous studies showed that the microbial biomass of organic-inorganic treatment was significantly higher than that of single chemical fertilizer treatment under the condition that the amount of N, P and K were equal [36,37]. Wang et al. found that the increase of SMBP was more significant by applying organic fertilizer alone [38]. In our study, the highest SMBC, SMBN, and QMB values of 75% OSR and the highest SMBP of 100% OSR indicated that the higher OSRs can maintain the soil biological fertility more effectively under the recultivation of long-term abandonment agricultural soil.

In general, the appropriate OSRs can not only ensure crop yield and quality but also improve soil fertility to a certain extent. Our results showed that the 100% chemical fertilizer (CF treatment) or 25% OSR (M25 treatment) can obtain higher vegetable yield. With the increase of OSRs, the quality of vegetable and soil had a significant increase. PCA of microbial and physicochemical properties indicated that M50 and M75 treatments were the optimal substitution rate for the maintenance of soil fertility in this long-term abandoned agricultural soil.

4 Conclusion

Under the same N application rate, the single application of chemical fertilizer (CF treatment) had the highest Chinese cabbage and spinach yield (54.4 and 24.8 t hm⁻², respectively), but with the increase of OSRs, the vitamin C content of Chinese cabbage and spinach significantly improved. Compared with CF treatment, 50% and 75% OSRs significantly increased SP, MWD, SOC, SMBC, SMBP, catalase activity, and urease activity. PCA of soil microbial and physicochemical properties under
different OSRs indicated that 50% and 75% OSRs were more effective for the maintenance of soil fertility in this long-term abandoned soil. After taking into consideration the vegetable quality and soil fertility, 50% and 75% OSRs should be recommended in practice for the optimal substitution rate. In future, long-term observation and research should be carried out in different idle and abandoned soils, as well as under different climate and environmental conditions.

Disclosure statement

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