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Effect of Rural Black-Gray Water Treatment by Subsurface Wastewater Infiltration System on Soil Environment of Vegetable Crop Field

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Abstract: A field trial was conducted in Tianjin to assess the impact of “three-compartment septic tank (SPT) + soil wastewater infiltration system (SWIS)” on vegetable crop soil, determine the SPT effluent quality, and establish the consumer safety of vegetables grown above the SWIS. The effluent total nitrogen (TN), total phosphorus (TP), ammonium-N ($\text{NH}_4^+\text{-N}$), chemical oxygen demand (COD), and 5-d biochemical oxygen demand (BOD_5) levels all varied largely every month. The average COD failed to meet the criteria of the *Standard for Irrigation Water Quality* (No. GB5084-2021) but significantly influenced bacterial community distribution. Hierarchical clustering disclosed seasonal variation in SPT effluent. SWIS treatment of rural black-grey water significantly affected both the vegetable soil TN and TP content, and it promoted microbial community diversity and richness in deep soil. The treatment also increased the relative abundances of the beneficial bacterial genera *Thiobacillus* and *Arthrobacter* by more than 320% and decreased the relative abundance of the pathogenic bacterial genus *Streptomyces* in vegetable soil by more than 20.33%. The faecal coliform levels and ascaris egg mortality rates in the vegetable crop soils lay within published human health and safety thresholds both before and after SWIS treatment. All vegetable crops grown above the SWIS were fit for human consumption. The VC level in the vegetables planted in experimental households were higher than those for the vegetables planted in ordinary households. The present work provides reasonable theoretical and empirical bases for optimising the “SPT + SWIS” process and SPT discharge standards in rural areas.

Keywords: effluent quality; soil environment; soil wastewater infiltration system; three-compartment septic tank; vegetable crop



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1. Introduction

Toilet systems are vital sanitary facilities and characteristic of advanced civilisations [1]. The Third Agricultural Census of China reported that only 48.6% of all rural households in China were equipped with sanitary toilets whereas 2% of them lacked toilet access altogether. Therefore, rural toilet transformation is an important step in the process of improving the rural environment in China. Toilet facilities popularised during the transformation in rural China mainly include three-compartment septic tanks (SPT), double-pit alternates, and double-vault funnels [2]. However, SPTs dominate the rural “toilet revolution” of China as they operate on a simple principle, are rapidly constructed, are comparatively safe and cost effective, and generate high-quality fertiliser [3]. However, the improper treatment of SPT effluent could result in contamination of the ambient environment [4].

SPT effluent is either returned to the field [5] (local fertilisation) or subjected to centralised clearing (unified transportation), and is transported by underground pipes to a nearby centralized sewage treatment station. The latter is applicable mainly in suburban or

rural areas with relatively better economic conditions. Hence, its practical use is limited [6]. The subsurface wastewater infiltration system (SWIS) [7] is a novel approach towards the disposition of SPT effluent. It has attracted a great deal of attention in the rural areas of China as it is characterised by low energy consumption, high efficiency, and simple operation, and it harmlessly treats the pollutants in the SPT effluent [8,9]. Yang et al. [7] built laboratory-scale SWIS to treat rural domestic sewage. The result showed that SWIS achieved a good chemical oxygen demand (COD) removal rate (>75%) and total phosphorus (TP) removal rate (>95%).

The SWIS directs SPT effluent into the deeper layers of soils with specific structure (detailed in Text S2) and permeability. The SWIS capitalises on the self-purification and self-regulation capacities of natural “soil-microorganism-plant” systems to eliminate the pollutants in effluent via a series of physical, chemical, and biological reactions [10]. However, the SWIS fails to recover the nitrogen and phosphorus in SPT effluent. Thus, these valuable plant macronutrients and others are wasted. Crops planted above the SWIS absorb the nutrients in the SPT effluent [11], reduce the SWIS pollutant pressure (excessive concentrations of pollutants may cause blockage of the SWIS system or plant maturity delay), and improve its economic benefits. However, it is as yet unknown whether this approach is feasible for raising safe and nutritious vegetable crops. Numerous scholars have studied the SWIS in recent years [12]. Most prior research has concentrated on pollutant removal and the rates at which greenhouse gas (GHG) emissions from SWIS are mitigated. Nevertheless, earlier work ignored the impact of SWIS operation on the ambient soil environment. Furthermore, Poustie et al. [13] confirmed that the application of SPT effluent may have beneficial or deleterious effects on the receiving soil-crop system and implications for human health. Therefore, it is important to know about the soil microbial functional after SWIS treatment in the long term.

In recent years, the condition of the rural aquatic environment in China has worsened as the untreated domestic sewage volumes have increased [14]. In addition, the domestic sewage in rural China has always been managed in accordance with the “*Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants*” (No. GB18918-2002) [15]. However, domestic sewage collection and discharge differ between rural and urban China, which has worsened the rural aquatic environment seriously. Moreover, rural villagers may lack awareness or education regarding environmental protection and dispose of large volumes of inadequately treated and potentially contaminated black-grey water (refer to SPT effluent in this article) [16]. Therefore, the quality characteristics of SPT effluent (rural black-grey water) must be established and a scientific basis must be provided to formulate SPT effluent discharge standards.

Based on the findings and limitations of the foregoing research, the present study combined SPT with the SWIS and planted and grew various vegetables above the latter over two seasons, and we hypothesized that the treatment is beneficial to the soil environment and that the vegetables are safe for humans. The specific aims and objectives of this study were to: (1) explore the effects of SWIS treatment of rural black-grey water (SPT effluent) on vegetable soil environment; (2) evaluate the safety and efficacy of planting vegetables crops above the SWIS; and (3) identify the SPT effluent quality characteristics and provide a scientific basis for optimising the “SPT + SWIS” process.

2. Materials and Methods

2.1. Overview of the Experimental Area

The study site was located in Zhang Laoren Village (39°44′ N and 117°49′ E), Dongjituo Town, Ninghe District, Tianjin, China (Figure S1). It had no specific SPT effluent treatment facilities that could contaminate the ambient environment. The background values (BJ) of the soil physicochemical indices, the hygienic index, and the SPT effluent quality indices are shown in Tables S1 and S2, respectively. The daily pollutant load per unit SWIS area is shown in Table S3.

2.2. Experimental Design

The “SPT + SWIS” technical mode was designed in accordance with the terrain of Zhang Laoren Village (Text S1).

2.3. SWIS Setup

“SPT + SWIS” was constructed outside the courtyard of a farmer (two adult and a child) in Zhang Laoren Zhuang, Ninghe District, Tianjin, China (Figure S2) on 13 July 2020. Details of the SWIS construction are shown in Text S2.

2.4. Analytical Methods

2.4.1. Sample Collection

Water samples (SPT effluent) were taken every other month between July 2020 and July 2021, and were designated H_1–H_13, respectively. The samples were collected in triplicate in sterile 500-mL bottles. One replicate was used to determine routine physicochemical indices, including ammonium-N (NH_4^+ -N), nitrate-N (NO_3^- -N), total nitrogen (TN), TP, COD, and 5-d biochemical oxygen demand (BOD_5) [17]. Another was used to detect the microbial community structure. Immediately after collection, each sample was drawn by vacuum through a filter membrane, which was then placed in a sterile tube and stored at -80°C until subsequent analysis [18]. The H_0, H_1, and H_13 were used for α diversity analysis, and H_1–H_13 were used for species composition analysis, hierarchical clustering analysis, and RDA analysis.

The soil samples were divided into two seasons. The first season was from July 2020 to March 2021 with four treatments (no fertilization): blank control group (CK), Chinese cabbage group (BC), cucumber group (HG), and radish group (SLB). The second season was from April to July 2021 with five treatment groups (fertilization): blank control group (CK), leek fertilization group (JC1), leek non fertilization group (JC2), cucumber fertilization group (HG1), and cucumber non fertilization group (HG2). Topsoil (0–20 cm) and deep soil (20–40 cm) samples were randomly collected from each treatment group once a month on average. The topsoil and deep soil samples of the four treatment groups taken in July 2020 were designated CK1_1 and CK2_1, BC1_1 and BC2_1, HG1_1 and HG2_1, and SLB1_1 and SLB2_1, respectively (_1 means the soil in July 2020). The topsoil and deep soil samples of the four treatment groups taken in March 2021 were designated CK1_9 and CK2_9, BC1_9 and BC2_9, HG1_9 and HG2_9, and SLB1_9 and SLB2_9, respectively (_9 means the soil in March 2021). The designated five treatment groups in the second season are detailed in Text S3. All of the other soil samples had similar nomenclatures. Vegetable samples were harvested in November 2020.

2.4.2. Testing Methods

The testing standards and methods used on the water samples are listed in Table S4. Fresh soil samples were divided into three lots. The first lot was used to determine soil TN and TP. The samples were stored and naturally air-dried at room temperature and passed through a 100-mesh sieve (the specific test method is detailed in Text S3). The second lot was sent to PONY Testing International Group to determine its hygienic indices, including faecal coliform levels and ascaris egg mortality rates. The third lot was placed in sterile 50-mL tubes and stored at -80°C until subsequent microbial community analysis.

Vegetable samples were harvested and sent to PONY Testing International Group to determine their pH and nitrite, vitamin C (VC), iron, reducing sugar, faecal coliform, and soluble sugar content, and to compare them against published national food safety standards (Table S5).

All physicochemical properties and hygiene data in this experiment were analyzed by using one-way ANOVA and Duncan multiple interval test in SPSS Statistics v. 26.0 (IBM Corp., Armonk, NY, USA). The least significant difference (LSD) test was used to identify significant differences among treatments ($p < 0.05$). All tables were plotted in WPS

Office 12.1.0.15120, and all images were drawn with Origin 2018 64-bit 9.50.00 (OriginLab, Northampton, MA, USA).

2.4.3. Health Risk Assessment

Soil hygiene indicators should be referred to as *Hygienic requirement for harmless disposal of night soil* (GB7959-2012) and *Hygienic specification for rural household latrine* (GB19379-2012); The faecal coliform levels in vegetables should be referred to as *Microbiological Examination of Food Hygiene-Enumeration of Coliforms* (No. GB4789.3-2016).

2.5. Microbial Community Analyses

2.5.1. DNA Extraction and PCR Amplification

Total bacterial DNA was isolated from both water and soil samples using E.Z.N.A.[®] Soil Extraction Kits (Omega Bio-Tek, Norcross, GA, USA) and measured by UV-Vis spectrophotometry (Bio Photometer; Eppendorf GmbH, Hamburg, Germany). DNA quality was determined by 0.9% agarose gel electrophoresis. The target primers were 338F (5'-GCACCTAACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR amplification and library construction were performed according to Liu et al. [19]. The PCR products were sequenced in triplicate on the Illumina Miseq PE300 platform (Illumina, San Diego, CA, USA) at Major Bio-pharm Technology Co., Ltd., Shanghai, China. Chimeras were removed, the sequences were extracted, and a 97% similarity threshold was adopted to cluster identical operational taxonomic units (OTUs) [20].

2.5.2. Microbial Diversity Analysis

The Ace, Shannon, and Coverage alpha diversity indices were determined according to the OTU levels [21]. Microbial community histograms and heatmaps were plotted to identify the relative changes in phylum- and genus-level bacterial abundances among the samples. Hierarchical clustering (based on the Bray-Curtis distance algorithm) was used to reflect the relationship between each sample. A redundancy analysis (RDA) was used to reflect the relationships among the bacterial taxa and the environmental factors.

3. Results and Discussion

3.1. Characteristics of SPT Effluent Quality

3.1.1. Physicochemical Indexes

Changes in the physicochemical indexes of the SPT effluent are shown in Figure 1. The TN concentration (Figure 1a) was in the range 39.8 mg·L⁻¹ (H₄)–185 mg·L⁻¹ (H₁₃). The variation in TN declined from H₁ to H₄ and fluctuated from H₄ to H₁₂. The variation in NH₄⁺-N concentration (Figure 1c) was consistent with that of TN. This may be due to the fact that under anaerobic conditions, the NH₄⁺-N accounted for the most of the TN in the SPT effluent [22]. However, some of the NH₄⁺-N was gradually consumed and converted to NO₃⁻-N during transport (Figure 1d). For this reason, NH₄⁺-N comprised only a small proportion of the TN in the effluent.

The TP concentration (Figure 1b) was in the range 0.54–98.31 mg·L⁻¹ and its median was 18.94 mg·L⁻¹. The TP concentration decreased from 92.06 mg·L⁻¹ (H₂) to 0.81 mg·L⁻¹ (H₃). Thereafter, the TP concentration increased and then decreased. During this period, the TP concentration increased with decreasing temperature. Johnson et al. [23] found that in the range of 15–30 °C, the efficiency of biological P removal increases with ambient temperature, and at temperatures > 30 °C, the efficiency of biological P removal declines. However, the main reason for the increase in phosphorus concentration at low temperatures may be the decrease in water consumption [24]. The TP concentrations measured here were similar to those reported by Lowe et al. [25] for septic tank effluent, namely, range 0.24–33.05 mg L⁻¹ and median 10 mg L⁻¹. The high TP concentrations recorded for H₁ and H₂ may be explained by the relative increase in grey water discharge in summertime [26].

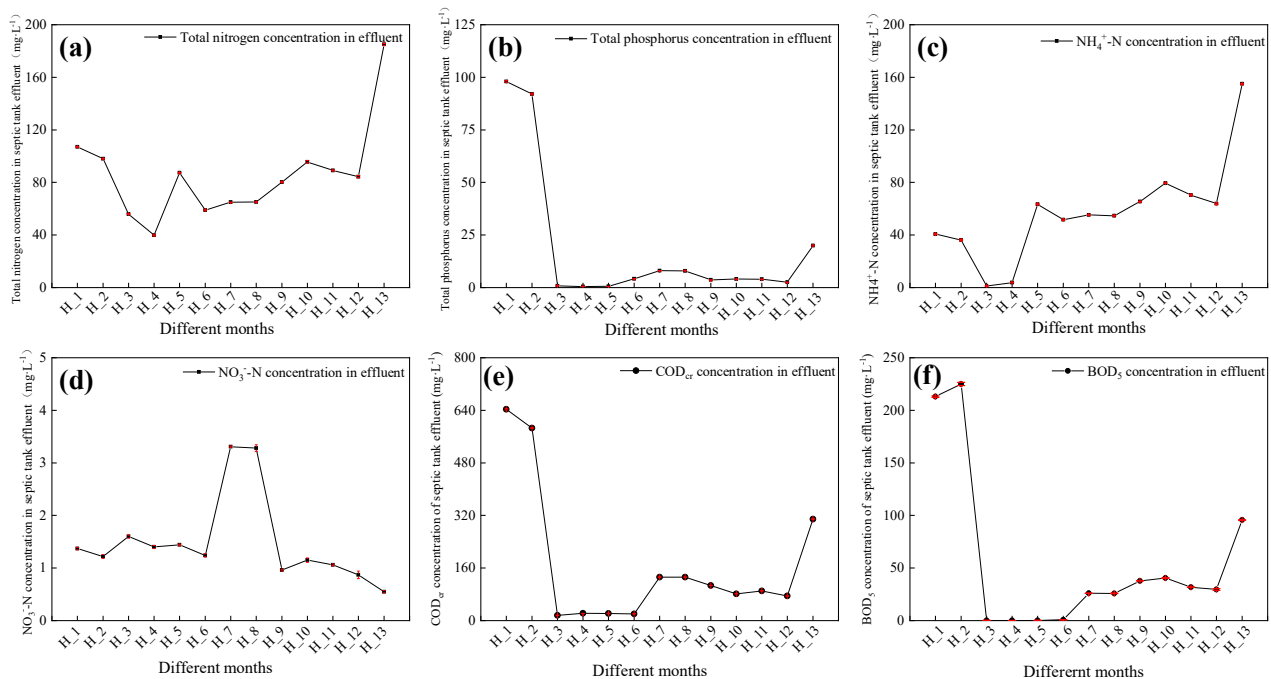


Figure 1. Variation trend of physicochemical properties of the SPT effluent. Note: H₁–H₁₃ represents from July 2020 to July 2021, respectively. (a) TN; (b) TP; (c) NH₄⁺-N; (d) NO₃⁻-N; (e) COD_{cr}; (f) BOD₅.

Chemical oxygen demand (COD) is an important aquatic environment quality indicator [27]. However, the average COD (Figure 1e) here was 172 mg L⁻¹ and it failed to meet the threshold published in the Chinese *Standard for Irrigation Water Quality* (No. GB5084-2021). Therefore, SPT effluent directly returned to the field could potentially harm the environment [28]. The concentration of BOD (Figure 1f) varies greatly, with a higher concentration at H₁ and H₂ but a sudden decrease at H₃, followed by a continuous upward trend. This may be due to the fact that the test household discharged livestock and poultry manure into the septic tank, which would result in a high BOD₅ concentration in the effluent of the septic tank.

3.1.2. Microbial Community

The alpha diversity of the microbial community in the SPT effluent is shown in Table 1. The Coverage index was 0.99 for all samples. Hence, the sequencing results might reflect the actual situation of the microbial community [29]. The Shannon and Ace indices were analysed for H₀, H₁, and H₁₃ to explore changes in microbial community diversity and richness, respectively [30]. Table 1 shows no significant differences among H₀, H₁, and H₁₃ in terms of their Ace indices ($p > 0.05$), but significant differences among them in terms of their Shannon indices ($p < 0.05$). Thus, the microbial community richness of the SPT effluent significantly changed over time and underwent seasonal variation.

Table 1. The SPT effluent H₀, H₁ and H₁₃ microbial community diversity index table.

Sample	Shannon	Ace	Coverage
H ₀	1.300 ± 0.045 b	30.152 ± 3.055 a	0.999 ± 0.000 a
H ₁	0.971 ± 0.057 c	38.944 ± 15.587 a	0.999 ± 0.000 a
H ₁₃	1.600 ± 0.017 a	36.557 ± 4.608 a	0.999 ± 0.000 a

Note: H₀, H₁, H₁₃ indicate SPT effluent in July 2020, August 2020, and July 2021, respectively, and the same below. Lowercase letters indicate significant differences between different treatments under the same diversity index ($p \leq 0.05$).

A Venn diagram (Figure 2a) was plotted to display the microbial core of the SPT effluent. Sixty-two operational taxonomic units (OTUs) were shared among the months in a single year. Therefore, all samples had similar growth environments [31]. The relative monthly phylum-level abundances of the SPT bacterial community are shown in Figure 2b. The dominant phyla in all samples were Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota, and Synergistota, and they accounted for >90% of the total reads [32]. Other main phyla included Campylobacterota, Desulfobacterota, Verrucomicrobiota, and Patescibacteria. Miao et al. [33] showed that Proteobacteria played dominant roles in biological denitrification and organic compound degradation. Vilela et al. [34] demonstrated that Proteobacteria predominated in sewage, followed by the seasonally variable Actinobacteria and Bacteroidetes. Here, Proteobacteria were relatively more abundant at lower temperatures. Their average relative abundances were 33% in winter (H_5, H_6, H_7), 28.4% in spring (H_8, H_9, H_10), 26.5% in summer (H_11, H_12, H_13, H_1), and 5.8% in autumn (H_2, H_3, H_4) [35].

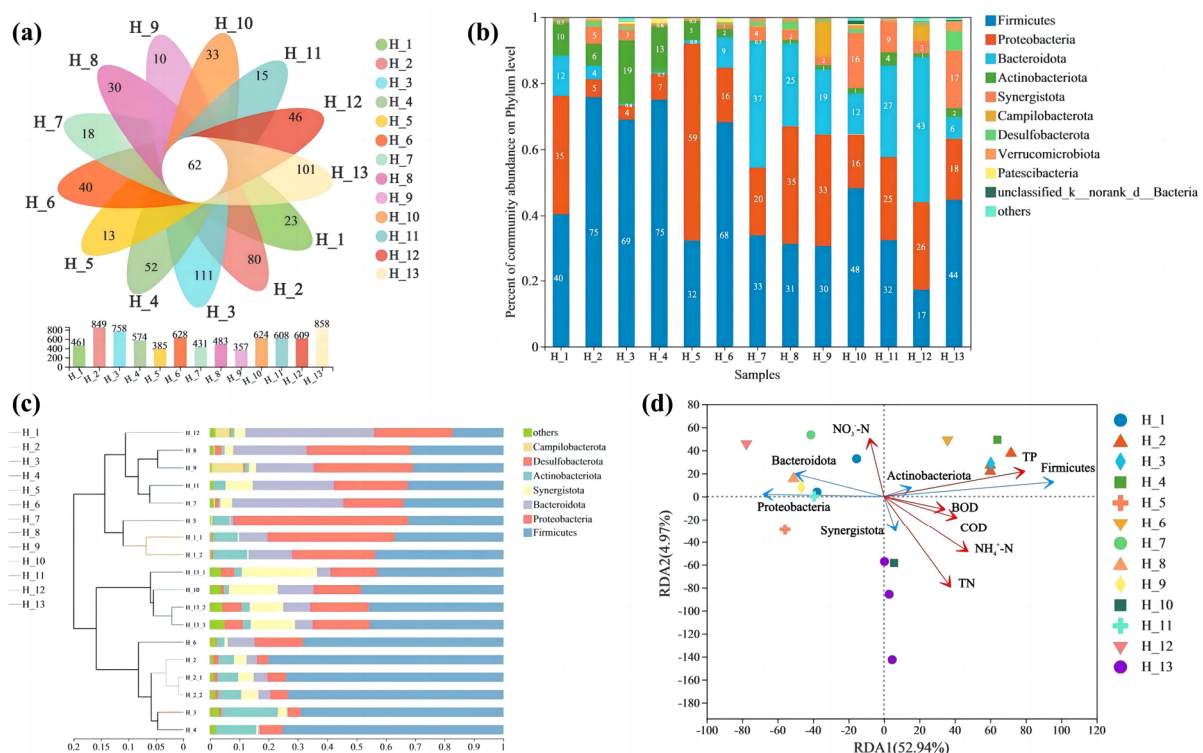


Figure 2. The SPT effluent: (a) Venn diagram at OTU level; (b) Compositional histograms of relative abundance of bacterial on phyla level (unit of numbers: percentage (%)); (c) Hierarchical clustering tree based on Bray-Curtis distances at phylum level; (d) RDA on phylum level.

Hierarchical clustering (Figure 2c) divided the 13 samples into four parts: (a) H_12, H_8, H_9, H_11, and H_7 (spring); (b) H_5 and H_1 (winter); (c) H_13 and H_10 (summer); and (d) H_2, H_3, H_4, and H_6 (autumn), and it revealed that the SPT effluent varied seasonally. Liu et al. [36] reported wide seasonal variation in the microbial community. The microbial community structures were significantly more similar within than among seasons. Wei et al. [37] demonstrated that seasonal change strongly influenced the bacterial community. The results of these studies were all similar to this study.

RDA (Figure 2d) was performed to disclose possible relationships among the microbial community and environmental factors (95% confidence interval, $p < 0.05$). RDA1 and RDA2 represented interpretations of 52.94% and 4.97%, respectively. TN and TP were the dominant factors affecting the microbial community. Firmicutes, Actinobacteriota, and Synergistota abundances were positively correlated with TP, TN, BOD, COD, and NH₄⁺-N, and were negatively correlated with NO₃⁻-N. Zhang et al. [38] reported that COD significantly

affected the microbial community distribution. A similar finding was made in the present study. Wirth et al. [39] reported that Actinobacteriota was effective in the degradation of complex organic compounds, although in this RDA, there was a positive correlation between Actinobacteriota and complex organic compounds, which verified the results of the previous study. However, the other dominant bacterium, Proteobacteria, was negatively correlated with the complex organic compounds, which can be attributed to the facts that the rural households locations were scattered and the residents had different farming and rest times, resulting in the large fluctuations in sewage quality and quantity [40]. Hence, Actinobacteriota plays an important role in the removal of organic pollutants in rural SPT.

3.2. Effects of SWIS Treatment of Rural Black-Grey Water on the Vegetable Garden Soil Environment

3.2.1. Soil Physicochemical Properties

The TN levels (2020) in the different soil layers of the four treatment groups are shown in Figure 3. During the test period, the TN levels in the topsoil and the deep soil generally increased and then decreased under all treatments. The TN levels under CK, HG, and SLB reached their maximum in November (CK_5, HG_5, and SLB_5). There were relative increases in the amounts of faecal sewage discharged in November. Consequently, the sewage pollutant loads and, by extension, the amount of nitrogen adsorbed by the soil increased [41]. For the vegetable treatment groups (BC, HG, and SLB) between sowing (July 2020) and harvest (November 2020), the topsoil TN content increased by 589.77%, 356.93%, and 70.00%, respectively, and the deep soil TN content increased by 266.46%, 718.22%, and 148.70%, respectively. SWIS treatment of rural black-grey water increased the TN content in both the surface and the deep soils of garden vegetable fields. Compared with the TN level in the absence of SWIS treatment of rural black-grey water (Figure S3), the TN levels significantly differed between soil layers under all treatments, except the deep soil of BC ($p < 0.05$). Therefore, the SWIS treatment of rural black-grey water significantly influenced the TN content in both vegetable soil layers.

In the second season, the topsoil TN levels (Figure S4a,b) were higher for the fertilisation groups (JC11 and HG11) and CK1 than the non-fertilisation groups (JC21 and HG21), which was similar with a previous study [42]. However, the topsoil TN levels for the non-fertilisation groups (JC21 and HG21) were lower than that of CK, possibly due to root absorption under the SWIS treatment [12]. Wang et al. [26] also found that from 0 cm to 60 cm depth, the cropland TN levels decreased regardless of the fertilisation treatment.

The TP levels in the various soil layers of the four treatment groups are shown in Figure S5. Overall, the TP content under each treatment varied little over different months. Under all treatments, the soil TP levels initially increased, then decreased, and then increased. From July 2020 (sowing) to November 2020 (harvest), the TP content increased under all treatments. For the BC, HG, and SLB treatments, the TP content of the topsoil was higher than that of the deep soil in November. This may due to the fact that the phosphorus in the rural black-grey water was absorbed and utilised by the roots after adsorption, precipitation, and microbial assimilation by the SWIS, and accumulated in the surface soil in the form of organic residues [43].

Between November 2020, and February 2021, the topsoil TP content declined under all four treatments. As the temperature rose from March onwards, the TP content significantly increased in both soil layers under all four treatments. Phosphorus migration in the soil might be temperature sensitive. Soil phosphorus adsorption may increase with temperature [44]. Compared with the TP level of the soil not subjected to the SWIS-treated rural black-grey water (Figure S6), the TP levels significantly differed between soil layers under the other treatments ($p < 0.05$). Hence, the SWIS treatment of rural black-grey water significantly influenced the TP content in both vegetable soil layers.

We then sought to determine whether the SWIS treatment of rural black-grey water affects fertilisation and non-fertilisation in both vegetable field soil layers. We analysed the differences between both of the soil layers of each treatment group in 2021. For CK,

JC1, and HG1, the topsoil TN levels (Figure S7a) were higher than those of the deep soil. Thus, the SWIS treatment of rural black-grey water combined with fertilisation increased the topsoil TN content, which, possibly due to the N-functional microorganism, preferred to be enriched on the topsoil under the “SWIS treatment + fertilisation” [45].

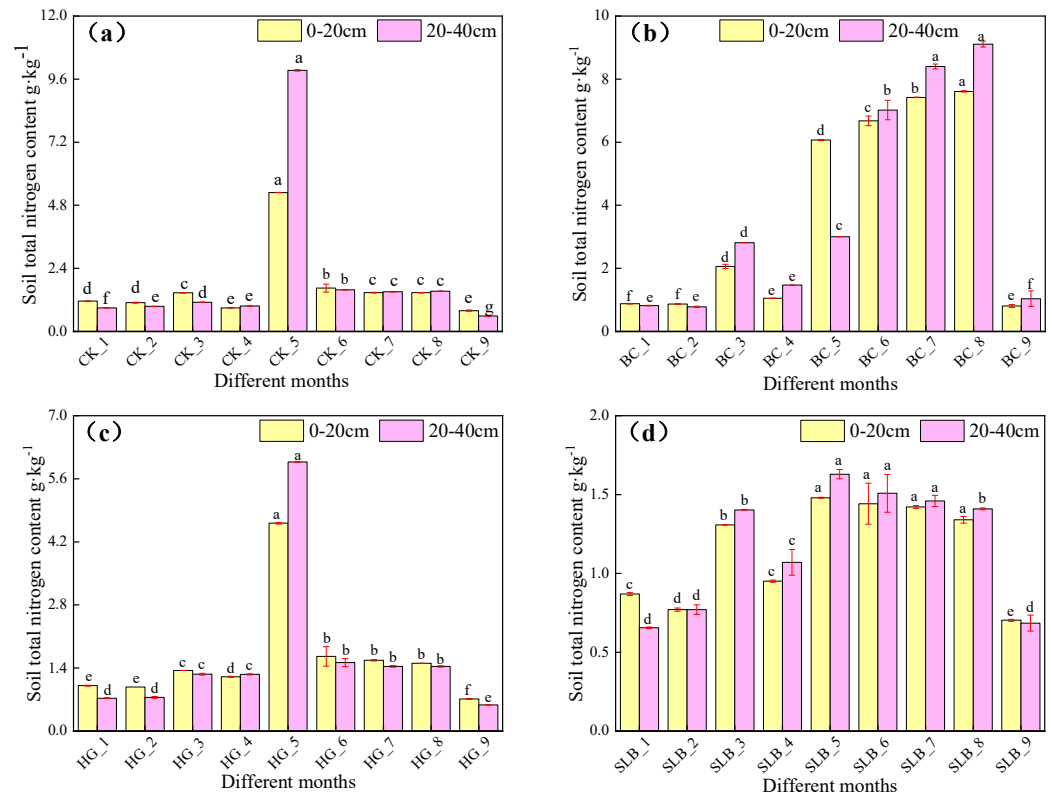


Figure 3. Changes of soil total nitrogen content in four treatment groups of vegetable fields in 2020: (a) CK group, (b) BC group, (c) HG group, (d) SLB group, which are the same as below. Numbers 1–9: July 2020–March 2021, respectively. Different lowercase letters in a column indicate significant differences at the 0.05 level.

3.2.2. Soil Microbial Community

Table 2 shows the alpha diversity indices for the topsoil and deep soil microbial communities in each vegetable field treatment group in 2020. For the topsoil, only the Shannon, Ace, and Coverage indices of the BC group were lower than those of BJ in March. The Shannon indices of the other three treatment groups were higher than that of BJ, and the difference was significant ($p < 0.05$). However, the Ace indices did not significantly differ among treatments ($p > 0.05$). For the deep soil, all three of the diversity indices in all of the treatment groups were higher than those of BJ in March, and the differences were significant. Hence, the SWIS treatment of rural black-grey water promoted microbial richness and diversity in the deep soils, but it had no significant effect on bacterial community richness in the surface soils of the vegetable fields. Cao et al. [46] demonstrated that irrigation can significantly increase soil surface microorganisms, and this is contrary to the results of this study. This indicates that irrigation and SWIS have significantly different impacts on soil microbial communities in different soil layers. SWIS treatment and crop cultivation can increase plant root exudates in deep soil that result in the accumulation and increase in organic C, which ultimately leads to an increase in soil microorganisms and their functional diversity [47].

Table 2. Soil microbial community diversity index table of each soil layer in each treatment group of vegetable field (2020).

Indexes Treatment	Shannon		Ace		Coverage	
	Top Soil	Deep Soil	Top Soil	Deep Soil	Top Soil	Deep Soil
BJ	6.184 ± 0.13899 b	6.2434 ± 0.0657 b	3758.57 ± 428.34 a	3051.90 ± 114.92 c	0.956 ± 0.002 a	0.964 ± 0.000 a
CK	6.607 ± 0.05961 a	6.5576 ± 0.0395 a	4017.65 ± 298.15 a	4126.78 ± 314.93 a	0.952 ± 0.001 a	0.952 ± 0.001 b
BC	5.945 ± 0.21989 c	6.6366 ± 0.0132 a	3658.23 ± 151.71 a	4555.95 ± 112.86 a	0.956 ± 0.001 a	0.949 ± 0.001 b
HG	6.713 ± 0.09131 a	6.5482 ± 0.1132 a	3945.15 ± 324.38 a	3781.61 ± 224.30 b	0.948 ± 0.001 b	0.953 ± 0.002 b
SLB	6.493 ± 0.09850 a	6.5414 ± 0.0661 a	3944.56 ± 216.07 a	3702.72 ± 181.45 b	0.952 ± 0.001 a	0.951 ± 0.002 b

Note: BJ: soil before infiltration treatment; CK: the blank control group; BC: the cabbage group; HG: the cucumber group; SLB: the summer radish group. Different lowercase letters in a column indicate significant differences at the 0.05 level.

Table S6 shows the soil microbial community diversity indices for each soil layer in each vegetable field treatment group (2021). The Shannon and Ace indices in the fertilisation group increased in each soil layer from April to July. However, only the topsoil Shannon and Ace indices of CK decreased between April and July. Therefore, the bacterial community diversity and richness increased under the SWIS treatment of rural black-grey water combined with fertilisation, which could be forecasted owing to the suitable soil-micro environment for the growth of microorganisms by the SWIS treatment and fertilisation. Guo et al. [48] reported that claimed water and middle N treatments could enhance soil microorganism richness, which is similar to the results of this study. Hence, it is a good strategy to plant crops on SWIS. Furthermore, the use of organic fertilisers may increase the soil organic matter (SOM) content [42], thereby stimulating bacterial community growth and diversity [49].

The relative genus-level abundances of the microbial community are shown in Figure 4. A heatmap revealed that the significant genus-level differences in soil microbial community were closely associated with vegetable type and fertilisation. Of the 30 relatively abundant bacterial genera, *Arthrobacter*, *Gaiella*, *MND1*, *Sphingomonas*, *RB41*, and *Thiobacillus* were abundant in both the topsoil (Figure 4a) and the deep soil (Figure 4b) of each vegetable field treatment group (2020). In 2020, *Arthrobacter* and *Streptomyces* were enriched in the topsoil (Figure 4a), whereas *Thiobacillus* and *Lysobacter* were depleted in all of the treatments relative to BJ. Compared with the deep soil of BJ, (Figure 4b), the relative abundances of *Thiobacillus* were 320%, 1006.6%, 1573.3%, and 3813.3% higher in the deep soils of CK2, BC2, HG2, and SLB2, respectively. By contrast, relative to the deep soil of BJ, the abundances of *Streptomyces* were 20.76%, 20.33%, 35.16%, and 48.30% lower in the deep soils of CK2, BC2, HG2, and SLB2, respectively.

Arthrobacter is a Gram-positive bacterium, and is ubiquitous in air, water, and contaminated soil as well as on human skin. Nakajima [50] indicated that *Arthrobacter* strains play key roles in polluted water treatment and heavy metal adsorption in the soil. *Thiobacillus* is a common colourless bacterium in soil and water that furnishes plants with the essential macronutrient sulphur. *Lysobacter* spp. are antagonistic to fungi, Gram-negative- and Gram-positive bacteria, and nematodes. They have unique sliding and bacteriolytic properties, and they may be highly useful for biological pest control. However, the potential human health risks associated with *Lysobacter* spp. remain to be determined [51]. Pathak et al. [52] demonstrated that *Thiobacillus ferrooxidans* and *T. thiooxidans* remove and leach heavy metals from the environment and facilitate the ore smelting process. However, *Streptomyces* is a potential plant pathogen [53]. Overall, SWIS treatment of rural black-grey water increases the relative abundance of environmentally beneficial genera, such as *Thiobacillus* and *Arthrobacter*, while simultaneously reducing those of microbial pathogens, such as *Streptomyces*, in deep vegetable field soils.

consistent with the results of Wei et al. [59] and Tang et al. [60]. Moreover, we also found that pH significantly influenced the vegetable field soil environment in both 2020 and 2021, and that it was significantly ($p < 0.05$) associated with the microbial community.

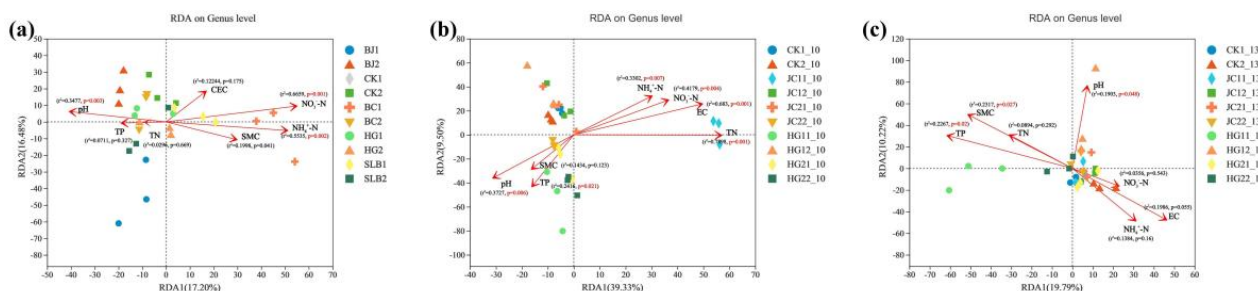


Figure 5. RDA of the relationships between microbial community and soil physicochemical properties: (a) vegetable soil (2020), (b) vegetable soil in April (2021), (c) vegetable soil in July (2021).

The soil layers of the fertilised (JC1, HG1) and non-fertilised (JC2, HG2) treatment groups were clearly differentiated after the April fertilisation treatment (Figure 5b), and all of the soil physicochemical indices except SMC ($r^2 = 0.1434$, $p = 0.123$) contributed to these differences. In July 2021 (Figure 5c), however, the soil microbial communities were separated only between HG11 and HG12. The microbial community might be unstable during this period [61]. Therefore, long-term monitoring is required to elucidate and model the responses of soil microbial communities to fertiliser amendments [62].

3.2.3. Soil Hygienic and Sanitary Properties

The hygienic and sanitary properties of the vegetable field background and treatment soil layers in 2020 are shown in Table S7. The faecal coliform levels in both soil layers of all treatments were $>>10^{-4}$, and ascaris egg mortality reached 100%. Hence, the faecal coliform levels met the thresholds of the Chinese standard *Hygienic Requirements for Harmless Disposal of Nightsoil* (No. GB 7959-2012). Moreover, the wet facility faecal coliform levels met the minimum requirements of the *Hygienic Specification for Rural Household Latrines* (No. GB19379-2012). In 2021, the faecal coliform levels were all >1.11 g and the ascaris egg mortality rate was 100% under all of the treatments. Thus, the SWIS treatment of rural black-grey water did not significantly alter the soil hygienic index of the garden vegetable field, and it maintained compliance with national sanitation and safety standards.

3.3. Effects of SWIS Treatment of Rural Black-Grey Water on Vegetable Harvest

We compared the safety and quality of vegetable crops raised on SWIS-treated soils against those of the same vegetables grown in ordinary households within the same village. The safety and quality indices are compared in Tables S8 and S9.

In 2020, the cabbage (BC), cucumber (HG), and water radish (SLB) grown in the experimental households had higher iron content than the same vegetables grown in the ordinary households (Table S8). The radishes grown in the experimental households also had higher Vitamin C (VC) content than those grown in the ordinary households. In 2021, the sugar content of the experimental household-grown BC and SLB, the iron content of the experimental household-grown BC, and the VC content of the experimental household-grown BC and HG were all higher than those of the same crops grown in the ordinary households (Table S9). Therefore, the nutritional value of the vegetables grown under the SWIS treatment was partially superior to that of the ordinary vegetables. Lu et al. [63] showed that reclaimed wastewater irrigation increased relative tomato yield by 10.3% and improved the fruit soluble sugar content without adversely affecting the VC or soluble solids content. The impact of the SWIS nutrients on crops may possibly depend on the SPT effluent pollutant load [64]. However, excess nitrogen in the effluent may delay plant maturity and weaken stems, while excess phosphorus in the effluent may

inhibit zinc uptake [65]. The levels of the foregoing nutrients may vary with septic tank construction specifications.

The faecal coliform levels in all vegetables grown in the experimental households (2020, 2021) and the ordinary households were all <3 MPN/g and met the threshold of the Chinese standard *Microbiological Examination of Food Hygiene-Enumeration of Coliforms* (No. GB4789.3-2016). No nitrite was detected in any vegetable harvested (2020, 2021). Overall, then, the vegetables grown on SWIS were safe for human consumption. Li et al. [66] detected a few *E. coli* cells on the leaves of asparagus lettuce irrigated with secondary sewage effluent. However, Farhadkhani et al. [67] used secondary wastewater and tap water in crop irrigation, and they found that both sources introduced *E. coli* to vegetables. In this case, the *E. coli* contamination might have originated from an external environmental source, such as birds or rodents [68]. Hence, the routine application of optimised SPT-SWIS in larger-scale vegetable crop cultivation is feasible.

4. Conclusions

The present study evaluated the influence of SWIS treatment of rural black-grey water on the physicochemical properties, microbial community abundance and diversity, and hygienic/sanitary characteristics of vegetable crop soil. We simultaneously assessed SPT effluent quality and the safety of vegetables raised above the SWIS. The changes in TN and NO_3^- -N content in the SPT effluent were similar. The monthly average COD failed to meet the published irrigation standard (No. GB5084-2021) and it significantly affected soil bacterial community distribution. The microbial community profile varied seasonally. SWIS treatment of rural black-grey water significantly influenced vegetable crop soil TN and TP content, promoted deep soil bacterial community alpha diversity, increased the relative abundances of the beneficial bacterial genera *Thiobacillus* and *Arthrobacter*, and decreased the relative abundance of the pathogenic bacterial genus *Streptomyces*. For soil hygiene index, the faecal coliform levels and the ascaris egg mortality rates met the criteria of the Chinese standards Nos. GB 7959-2012 and GB 19379-2012, respectively. The vegetables were safe and fit for human consumption, and they had certain nutritional value. Overall, the present study demonstrated that the “SPT + SWIS” process is beneficial to the soil environment in rural garden vegetable fields. This work provides a scientific basis for optimising the “SPT + SWIS” process and for ameliorating rural sewage discharge standards.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13092206/s1>, Figure S1. Location map of Zhanglaoren village. Figure S2. SWIS construction drawing. Figure S3. The TN content of each soil layer in each treatment group before and after SWIS treatment. Figure S4. (a) and (b) are the changes of TN in each soil layer at different periods, (c) and (d) are the changes of TP in each soil layer at different periods in 2021. Figure S5. Changes of soil total phosphorus content in four treatment groups of vegetable fields in 2020. Figure S6. The TP content of each soil layer in each treatment group before and after SWIS. Figure S7. The difference of (a) TN and (b) TP content between the two soil layers in each treatment every month. Table S1. Background values of soil physicochemical and sanitary indexes. Table S2. The effluent water quality index of the effluent of SPT (Before facility operation). Table S3. Daily of load pollutants in SWIS. Table S4. Test method and standard for the water quality index of the effluent of the SPT. Table S5. Vegetable sample index detection methods and standards. Table S6. Soil microbial community diversity index table of each soil layer in each treatment group of vegetable field (2021). Table S7. Hygiene properties of soil layers in each treatment group in vegetable field (2020). Table S8. Comparison of safety and quality indicators of vegetables grown by experimental households and ordinary households in 2020. Table S9. Comparison of safety and quality indicators of vegetables grown by experimental households and ordinary households in 2021. Text S1. Experimental design. Text S2. SWIS set up. Text S3. Designated five treatment groups in the second season. Text S4. Testing method of soil TN and TP.

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