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The Impact of Different Winter Cover Crops on Weed Suppression and Corn Yield under Different Tillage Systems

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Abstract: White clover (*Trifolium repens* L.) and ryegrass (*Lolium multiflorum* L.) are widely used cover crops. This experiment investigated the potential of white clover (WC), ryegrass (RG), and fallow (FL) to inhibit the growth of weeds and the effect of their residue return to the field on subsequent crops in a cover crop-corn rotation system. Furthermore, we designed pot experiments to guide the scientific application of WC and RG. The results showed that the FL treatment had the highest mean weed biomass in two years (11.99 t ha^{-1}) and the RG treatment recorded the lowest mean weed biomass in two years (2.04 t ha^{-1}) as its early growth rate and aerial root cover. The combination of rotary tillage (RT) and WC recorded the highest total corn yield in two years (20.20 t ha^{-1}) and an increase of 2.84% in the two-year average biomass of weeds compared to RT-FL. Compared to RT-FL, RT-RG treatments inhibited weed invasion by an average of 73%, but corn yield was reduced by 3.25%. Straw and soil ratios above 6:100 for RG resulted in stunted corn growth, including a reduction in fresh weight and chlorophyll content, and impaired photosynthesis, but this effect was not evident in WC treatment. From an ecological point of view, this study recommends RT-RG as a pre-crop for corn to reduce herbicide use.

Keywords: cover crop; conservation tillage; weed suppression; phytotoxicity; corn yield

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1. Introduction

In recent years, the widespread and excessive use of synthetic herbicides for weed control has led to many problems such as the development of herbicide-resistant weed species, damage to soil microecology, and environmental pollution. There is an urgent need to find other forms of weed control that can reduce the use of synthesis herbicides [1]. Crop rotation is an agronomic practice that combines land use and land nourishment and is an important method of soil management. The rotation of cash crops and some cover crops could significantly increase soil total nutrients, soil enzyme activities, and available phosphorus content and be beneficial for sustaining high crop yield [2]. In southern China, especially Guangdong Province, it is customary to alternately plant two cash crops and fallow the field in winter, which could be a waste of solar energy and land resources and could lead to erosion and nutrient loss [3]. Weed control in winter fallow fields is a difficult problem for conservation tillage. The ability of cover crops to prevent weed infestation is affected by their competitiveness and allelopathic mechanisms. It is a weed green control practice to control weeds by planting cover crops. Cover crops reduced the incidence of weeds, which is important for no-tillage sustainability [4]. Crop rotation, intercropping, cover cropping, and straw mulching are important allelopathic tools for green weed control technology [5]. Planting cover crops and using biotechnology to control weeds are currently hotspots of weed control research. Integrating with sorghum [*Sorghum bicolor* (L.) Moench] and sunflower (*Helianthus annuus* L.) extracts for weed control can reduce herbicide use by 75% [6]. Researchers found that subterranean clover (*Trifolium subterraneum* L.) can significantly reduce weed biomass and decreased the size of the soil seed bank and species richness [7]. These studies have shown that cover crop aqueous extracts can release phytotoxic substances that inhibit the growth of weeds. The weed growth was inhibited by cover crops with a variety of compound factors caused by common action. On the one hand, planting cover crops can control weeds by giving the crop a significant advantage [8–10]. On the other hand, the allelochemicals released by cover crops through natural decomposition, root secretion, and rain washing can inhibit the growth of weeds [8,11]. As green manure, *Eucalyptus globulus* leaves contain phenolic substances and volatile organic compounds that inhibit the growth of weeds [12]. *Ludwigia hyssopifolia* (G. Don) Exell has potential allelopathic activity and its leaf aqueous extract showed the highest phytotoxic activity, indicating its potential as a bioherbicide [13]. The most probable identity of the major phenolic compound is syringic acid. Studies have shown that intercropping red clover (*Trifolium pratense* L.) with winter wheat (*Triticum aestivum* L.) can inhibit the growth of weeds in soybean (*Glycine max* (Linn.) Merr.)-winter wheat-corn rotation mode. Planting red clover effectively suppressed weeds, the density of volunteer winter wheat, and seed production of downy brome by more than 99% compared with the control [14]. The above studies show that different tillage and crop rotation systems combined with cover crop cultivation and the release of phytotoxic substances from the decay of cover crops can effectively suppress the growth of weeds. Different types of cover crops have different effects on the growth of cash crops. Studies have shown that toxic compounds, such as diisobutyl-n-octylamine (DIBOA), released by rye (*Secale cereale* L.) stubble or aqueous extract of *Ludwigia hyssopifolia* (G. Don) can inhibit weed germination or growth processes of main crops [15–17]; however, some researchers found that both crop straws and cover crop straws could promote or remain neutral on crop growth [18,19]. These studies have shown that different receptor plants have different tolerances to specific phytotoxic effects.

White clover (WC) and ryegrass (RG) are multipurpose cover crops that are widely used in southern China. The objectives of this research were to better understand the effects of straw return on the growth of weeds and corn and to guide the scientific application of these two green manures. A two-year field trial was conducted in Guangdong, China. In the field experiments, we investigated the biomass of cover crops, corn yield, and the biomass of weeds during the corn growing period. To evaluate the possible side effects on corn, subsequent pot experiments were conducted to explore the effect of different cover crop straws and soil ratios on the germination and seedling growth of corn. It was hypothesized that different cover crops and tillage systems will significantly differ in weed control and corn yield. It was further hypothesized that less weed infestation will be noted for cover crops with phytotoxic crops, while the interaction effect of cover crops and tillage systems will improve the yield of corn. This research can help with using green manure scientifically, reducing the use of chemical herbicides, and improving the yield of corn.

2. Materials and Methods

2.1. Experiment Site Description

Field experiments were conducted at the experimental base of South China Agricultural University (23°14′18.42″ N, 113°38′8.06″ E) from 2019 to 2021. Soils were classified as Entisols, based on IUSS classification [20], and the 10 cm soil layer contained 40.67 $g \cdot kg^{-1}$ organic matter, 0.46 $g \cdot kg^{-1}$ total nitrogen, 0.70 $g \cdot kg^{-1}$ total phosphorus, 20.73 $g \cdot kg^{-1}$ total potassium, 49.61 $mg \cdot kg^{-1}$ inorganic nitrogen (sum of nitric and ammoniacal nitrogen), 102.09 $mg \cdot kg^{-1}$ available phosphorus and 78.59 $mg \cdot kg^{-1}$ available potassium. The experimental site used for field experiments had been dedicated to corn or other grain crops production for at least 20 years, with increasing weed infestation every year despite mechanical (rotary tillage and using the lawnmower) or chemical (glyphosate and paraquat) weed control. The study area is a subtropical monsoon climate, and the weather data for the crop periods of both years are given in Figure 1. The average temperature ranged from 6.5 °C to 32.5 °C during both years. Similarly, the relative humidity ranged between 36% and 100% during each study year. From November 2020 to early April 2021, there was little daily rainfall, and the test field was in a state of drought.

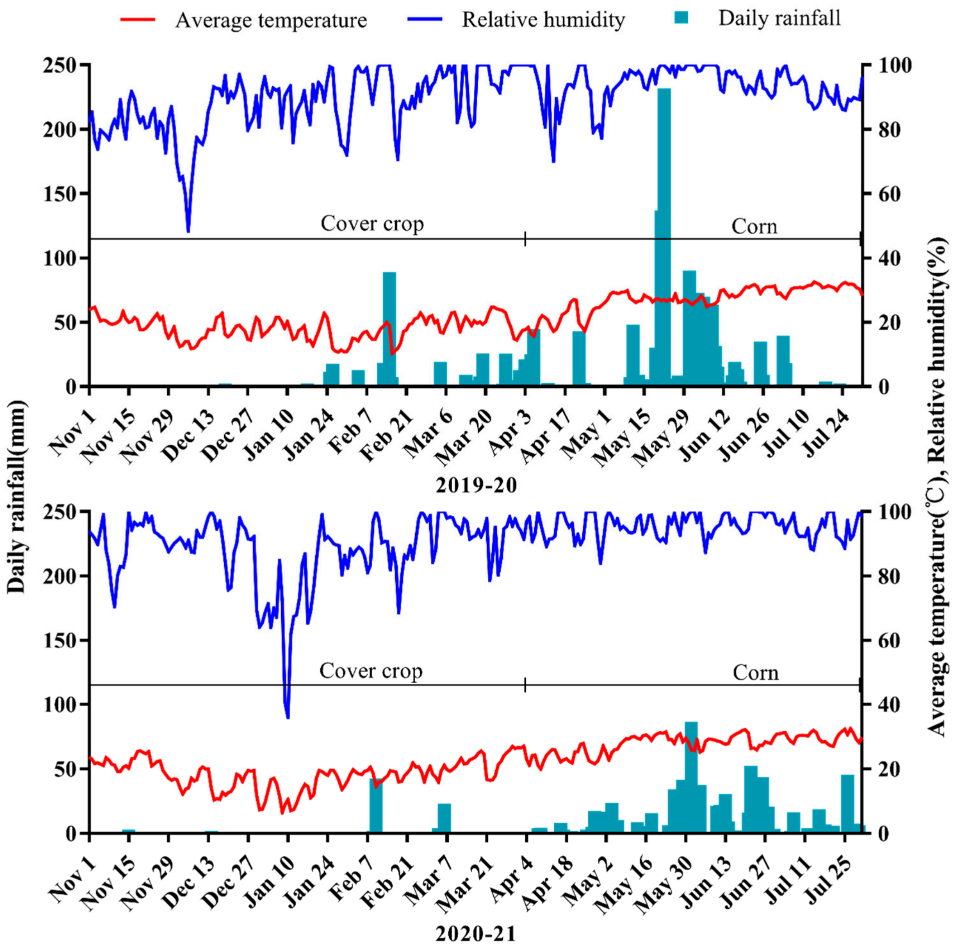

Figure 1. Weather data of the experimental site during cropping seasons of both years.

2.2. Experiment and Treatment Design

Field trials: The experimental design was split-plot with the main factors being tillage, no-tillage (NT), and rotary tillage (RT), and the split-plot factor was winter cover cropping (fallow (FL), which consisted of native weeds as a control, WC and RG). The main plot had an area of 4.2 × 9.7 m (40.74 m^2, excluding trenches), and contained three subplots (13.58 m^2). Four replicates were set up with a total of 24 plots, for a total plot area of 977.76 m^2. The distribution of the test field plots was detailed in Supplementary Figure S1.

Pot trials: In order to clarify the effect of different amounts of cover crop returned to the field on corn growth, we also designed a pot experiment to guide the scientific use of cover crops. The cover crop samples were air-dried for about 30 days under room temperature indoors to prevent ultraviolet light. Dried samples were ground into a fine powder and stored in the envelope bags before use. The dry weight yield of WC and RG were around 5000 kg ha^{-1} and the soil bulk density of about 1.3·g cm^{-3}. The tillage depths of no-tillage and rotary tillage were 1 and 6 cm, respectively. Thus, the straw and soil ratio (SSR) in the soil after cover crop return was about 0.6–5%. In practice, the amount of cover crop returned to the field can be adjusted by external inputs or internal removal, so the SSR is not fixed. Based on the above analysis, we used the dried powder of WC and RG as materials, mixed it well according to the mass ratio of powder: substrates (Jiffy®, consisting of peat soil, coconut bran, vermiculite, perlite) = 1:100, 3:100, 6:100, and 10:100, and put the mixture into flowerpots. Substrates without cover crops powder were used as control (CK). Each flowerpot was inoculated with nine corn seeds, and each treatment was set up with five replicates. The seeded pots were placed on a terrace where the average temperature during the experiment was 24 °C and the relative humidity was 85%. To ensure the authenticity of the results, all the management and environmental conditions were as consistent as possible.

2.3. Farming Manege

The cover crop was sown by hand in November (seed rate was WC (var: Huiya) 100 kg ha^{-1}, RG (var: Niuyi) 5.7 kg ha^{-1}), cover crop seeds purchased from Jiangsu Yujing Yuan Greening Engineering Co., LTD (Jiangsu, China). Cover crop biomass was collected manually at the end of March by sampling an area of 0.5 m × 0.5 m per plot and weighed immediately for biomass determination. Corn seeds (var: Black pearl waxy corn) were sown at the nursery site in mid-March at a rate of 30 kg ha^{-1}. The aboveground parts of the cover crops were mowed by a flail mower in early April. After the cover crop was mowed, tillage practice began. In RT, cover crop residues were also incorporated into the soil to a depth of 6 cm by a rotary cultivator. For the NT system, cover crop residues (including aerial roots) were left at the surface soil. After tillage, corn seedlings were transplanted into each plot at a density of 38,462 plants per hectare with rows separated by 0.65 m and plants within rows separated by 0.40 m. In this experiment, corn was manually harvested in early July. Aboveground corn residues were returned to the soil by a brush cutter. During the whole process of this rotation system, we used a hand push fertilizer machine to apply 30 kg fertilizer (Shidanli®, Shandong, China, 28N:13P:10K) at each corn tassel stage. Starting with tillage treatment, no subsequent weeding treatment was carried out until weed samples were collected to assess the impact of cover crops and tillage systems. We applied label dose herbicide (Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), Fengshan®, Jiangsu, China) once the weed survey was completed. To ensure the authenticity of the results, all management was as consistent as possible. We irrigated the corn every year after transplanting and conducted no other subsequent irrigation practices.

2.4. Crop Yield and Weeds Biomass

Cover crop biomass (cover crop yield) was collected manually at the end of March by sampling an area of 0.5 m × 0.5 m per plot. The fresh aboveground cover crop samples were weighed as the cover crop yield. Corn was harvested in early July by sampling five continuous uniform plants of corn per plot. The aboveground weight of the whole plant and the fresh weight of corncobs were weighed immediately for biological yield and economic yield. In addition, the morphological indicators of corn were determined. When the corn seedlings were transplanted and after one month, weed biomass was collected manually at ground level by sampling an area of 0.5 m × 0.5 m per plot, transferred to the laboratory for botanical characterization and classified into grasses, broad-leaved, and sedges, and then weighed. The sum of each species was the total weed biomass.

2.5. Data Collection

In the laboratory experiment, we determined the stem diameter, plant height, and fresh weight of second-leaf corn. Ten seedlings with uniform growth were selected from each treatment group to measure plant height, stem diameter, and fresh weight. The last fully expanded leaf of second-leaf corn seedlings from 5 replicates of each treatment was taken to determine the protective enzyme activity. SOD activity was measured by the NBT reduction method, POD activity was measured by the colorimetric method, and CAT activity was measured by the guaiacol method [21]. MDA content was measured by the TBA method, and the root activity was measured by the TTC method [22]. To measure the coverage and growth of green manure, we used CM 1000 NDVI instrument (Spectrum Technologies INC, Nebraska, USA) to measure the green manure NDVI value from sowing to harvest. Photosynthesis and fluorescence properties, i.e., net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (gtc), intercellular carbon dioxide concentration (Ci), instant water use efficiency (iWUE), maximal quantum yield of PSII (Fv/Fm), PSII actual photochemical quantum efficiency (φPSII), electron transport rate (ETR), photochemical quenching (qP), and nonphotochemical quenching (NPQ) were measured by an LI-6800 photosynthesis measurement system (LI-COR®, Lincoln, NE, USA). The last fully expanded leaf of 3 uniform corn seedlings at the fourth-leaf stage was selected from 5 replicates of each treatment to determine the photosynthesis and fluorescence properties. The chlorophyll content was expressed by the SPAD value, which was collected by SPAD-502 plus (Konica Minolta, INC., Tokyo, Japan). The last fully expanded leaf of a fourth-leaf corn seedling was selected from each of the 5 replicates of each treatment, and the SPAD value of the upper, middle, and lower parts of the leaf was measured, and their average value was calculated as the final SPAD value.

2.6. Statistical Analysis

Independent-samples *t*-test was used for different tillage methods. The data of different cover crops and pot experiments were analyzed by one-way ANOVA. The interaction between tillage and cover crop treatment was analyzed by two-way ANOVA. We verified normality and homogeneity of variance using Shapiro–Wilk test and Levene's test respectively. Significant differences were further compared using the post hoc Fisher LSD test. SPSS 24 was used for the significance analysis of the data at a significant level of 5%. GraphPad Prism 7 was used to plot.

3. Results

3.1. Corn Yield and Weed Biomass

After two years of field experiments consisting of tillage systems and cover crop planting, the experiment showed that different tillage methods and cover crop combinations had significant effects on corn yield and weed biomass. NT was more suitable for the growth of cover crops; in contrast, RT provided significant improvement in corn yield as well as weed biomass. The cover crop biomass in the first year was insignificant, but the biomass of WC was significantly improved in the second year. There was a significant change in the WC biomass between the two years because of frost, and WC show strong resistance to cold stress (Table 1).

Table 1. Effect of different tillage and cover crops on cover crop biomass, corn yield, and weed biomass [mean (SD), $n = 4$].

	Cover Crop Biomass (t ha^{-1})		Weed Biomass (t ha^{-1})		Corn Yield (t ha^{-1})	
	2020	2021	2020	2021	2020	2021
Tillage						
NT	8.26	17.09 a	7.63 b	5.78 b	3.98 b	7.69 b
RT	7.04	11.78 b	11.18 a	8.80 a	8.05 a	10.66 a
T	ns	**	*	**	***	**
Cover Crop						
WC	8.10	32.67 a	12.12 a	9.91 a	7.06 a	9.70 a
RG	9.10	7.88 b	2.24 b	1.84 b	4.82 b	7.82 b
FL	6.23	4.59 b	13.86 a	10.11 a	6.33 a	9.99 a
CC	ns	***	***	***	***	***
Interaction						
T × CC	ns	ns	***	ns	ns	***

1. T, tillage; CC, cover crop; NT, no-tillage; RT, rotary tillage; FL, fallow; WC, white clover; RG, ryegrass. 2. Numbers followed by different letters within the tillage, cover crop treatments, and their interaction effect was significantly different at $p < 0.05$ by LSD or Tamhane's T2 post hoc multiple comparisons respectively. Sig. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Considering the interaction effect of tillage methods and cover crops, corn yield, and weed biomass, the combination of RT and WC can ensure a stable and considerable corn yield as well as less weed biomass than FL. RT-RG recorded a 17.86 t ha^{-1} total yield of two years, which was close to the RT-FL18.46 t ha^{-1} (Figure 2). RG can significantly inhibit the total weed biomass and each species of weed. The total weed biomass in the RG treatment was the lowest, followed by WC. FL recorded the highest total weed biomass over the two years. Planting WC under NT farmland can reduce Gramineae biomass, but it might increase sedge weed biomass in the RT field. The inhibitory effect of WC on broadleaf weeds under the RT treatment was better than that under the NT treatment (Figure 3).

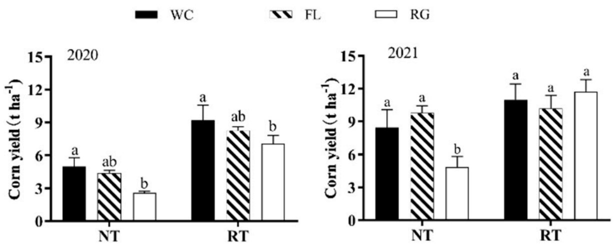

Figure 2. Corn yield over two years under different cover crops and tillage systems. NT, no−tillage; RT, rotary tillage. Different letters at the top of the bar chart denote significant differences among different treatments of cover crops for the same tillage at $p < 0.05$ by LSD test. The bar represents mean values of 4 replicates ± SD.

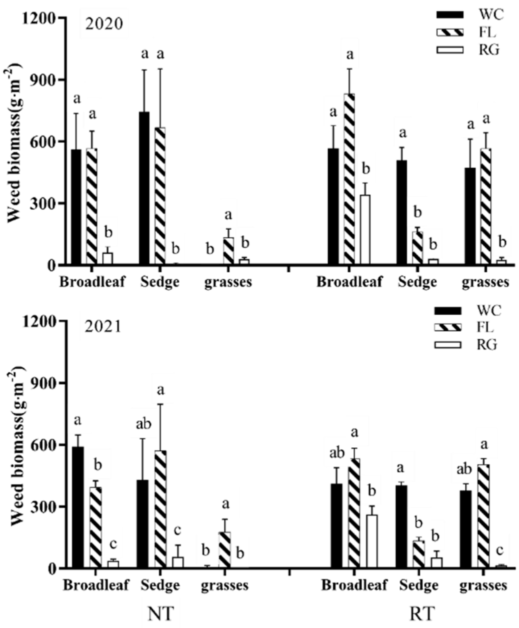

Figure 3. The impact of different cover crops and tillage methods on the biomass of weeds of different species. NT, no−tillage; RT, rotary tillage. Different letters at the top of the bar chart denote significant differences among different treatments of cover crops for the same tillage method and weed species at $p < 0.05$ by LSD test. The bar represents mean values of 4 replicates ± SD.

3.2. Continuous Monitoring of Vegetation Coverage after Cover Crop Sowing

There were several important time points above, i.e., 30 d, 40 d, 60 d, and 130 d, which indicated the entire population formation of RG, initial population formation of FL, initial population formation of WC, and the entire population formation of FL and WC, respectively. The early coverage of the RG plots was better in terms of NDVI values (Figure 4). WC gradually formed a population after two months of sowing. The vegetation coverage of RT-WC was higher than that of NT-WC at 70 d and 100 d. At 100 d after sowing, the vegetation coverage of WC surpassed that of RG, but the population was nonuniform.

At 130 d, the WC and RG vegetation coverage under the NT treatment was the highest among all treatments. RT-FL has a higher vegetation coverage than NT-FL. Vegetation coverage was consistent with the cover crop biomass. RG and WC planted in the NT field were better than those planted in the RT field based on the vegetation coverage. The biomass of RG aerial roots and underground roots was quite large. The RG aerial roots can completely cover the ground and prevent surface bareness (Figure 5).

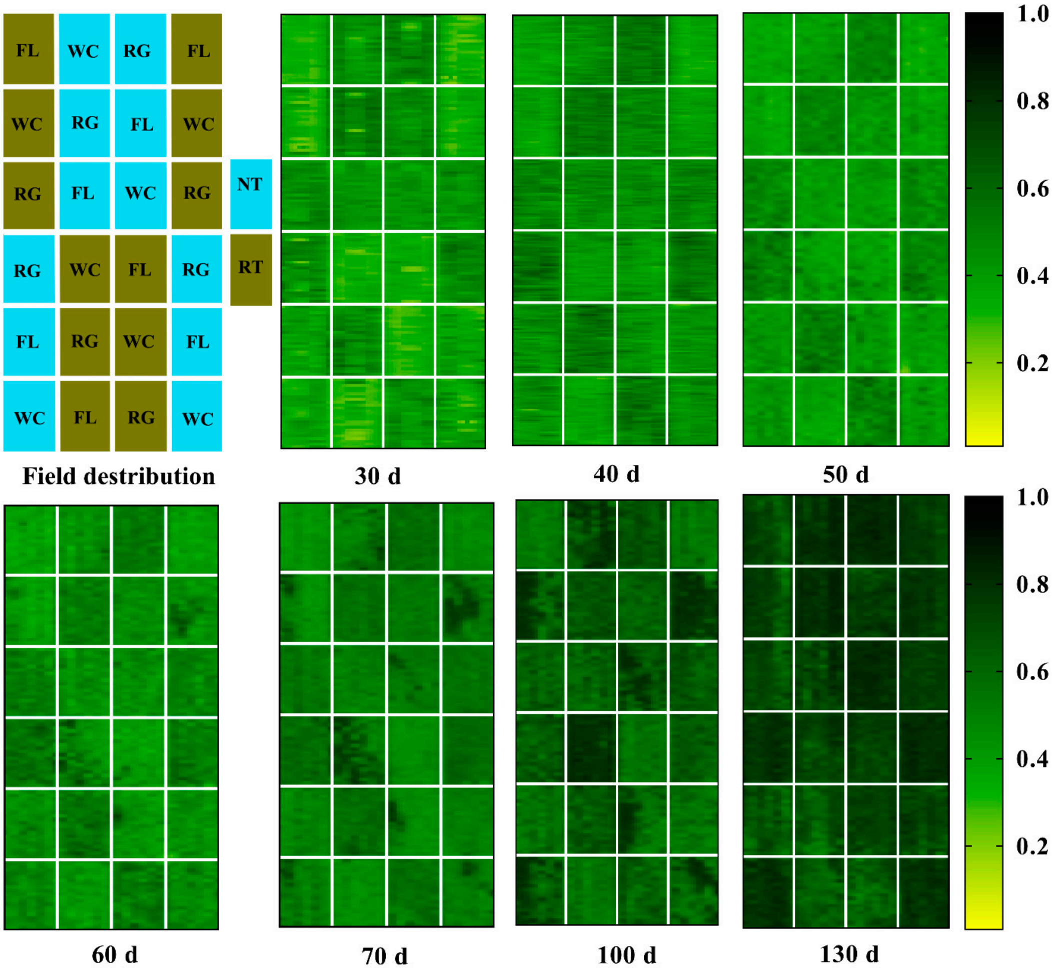

Figure 4. Change of vegetation coverage based on NDVI. NT, no−tillage; RT, rotary tillage; FL, fallow; WC, white clover; RG, ryegrass. Data was collected in different plots from 30 to 130 days after sowing.

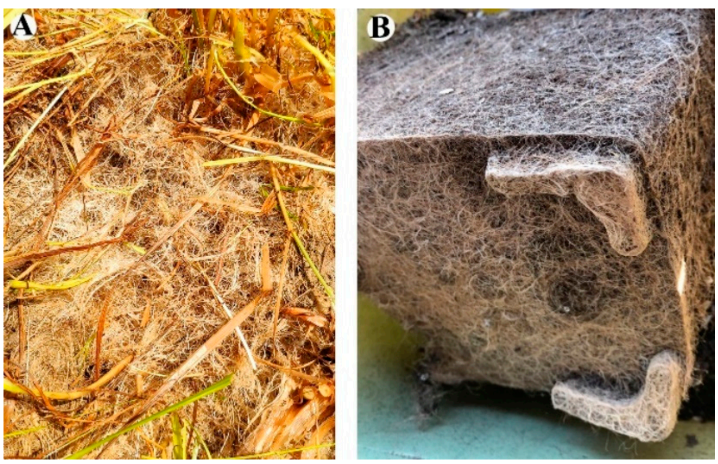

Figure 5. Covering effect of RG aerial root (**A**) and subsurface root (**B**).

3.3. Effect of Cover Crops and Tillage Systems on the Growth of Corn

All indicators except the economic coefficient were significantly affected by tillage methods, while cover crops significantly affected the stem diameter, panicle weight and SPAD of corn. The interaction effect of tillage systems and cover crops can significantly affect all the indicators except the barren ear tip of corn. In the RT systems, all the indicators of corn in the WC and RG treatments were better than those in the FL treatment except for the barren ear tip and economic coefficient. In the NT systems, all the indicators of corn in WC and FL were better than those in the RG treatment. In general, the RT system was more suitable for corn growth than NT (Table 2).

Table 2. Effects of different tillage methods and treatment of cover crop on physiological indexes of corn [mean (SD); n = 4].

Treatment	Plant Height (m)	Stem Diameter (mm)	Panicle Weight (kg)	Barren Ear Tip (mm)	SPAD Value	Economic Coefficient
NT-WC	1.25 (0.094) a	15.08 (1.84) b	0.22 (0.041) b	18.77 (3.02) ab	51.03 (2.12) b	0.56 (0.028) ab
NT-FL	1.27 (0.080) a	16.23 (1.09) ab	0.26 (0.017) ab	16.96 (2.50) c	54.31 (1.92) a	0.55 (0.013) ab
NT-RG	1.06 (0.10) b	11.37 (0.72) c	0.13 (0.025) c	23.54 (6.93) a	45.32 (2.82) c	0.55 (0.021) ab
RT-WC	1.30 (0.074) a	18.27 (1.18) a	0.29 (0.040) a	16.72 (2.35) c	55.40 (1.55) a	0.52 (0.024) b
RT-FL	1.25 (0.061) a	16.90 (1.17) ab	0.27 (0.033) ab	13.91 (3.56) c	55.08 (1.58) a	0.57 (0.024) a
RT-RG	1.35 (0.10) a	18.40 (2.07) a	0.29 (0.025) a	15.41 (0.53) c	56.00 (1.88) a	0.53 (0.039) ab
LSD value	T = 0.01, CC = 0.23 T × CC = 0.01	T = 0.00, CC = 0.04 T × CC = 0.00	T = 0.00, CC = 0.01 T × CC = 0.00	T = 0.01, CC = 0.12 T × CC = 0.24	T = 0.00, CC = 0.01 T × CC = 0.00	T = 0.36, CC = 0.23 T × CC = 0.07

1. T, tillage; CC, cover crop; NT, no−tillage; RT, rotary tillage; FL, fallow; WC, white clover; RG, ryegrass.
2. Numbers followed by different letters within each column were significantly different at $p < 0.05$ by LSD post hoc multiple comparisons.

3.4. Effect of Cover Crop Straw Returning Back to the Field on Growth of Corn

3.4.1. Straw Return on Seedling Morphological Properties

Cover crops returning to the field can significantly improve the root growth of corn in total length, surface area, branch points, and root tips in all the SSR treatments of WC and RG. In the SSR (≤3:100) of the WC treatment, it can improve the average diameter of corn, but the higher SSR of WC can cause a smaller average diameter of corn compared to the low SSR treatment (≤3:100). The RG straw significantly inhibited the average diameter of corn as the SSR exceeded 1:100. Both cover crops can increase the root volume of corn, among which the white clover treatment with a fertilizer soil ratio of ≤3:100 can increase the root volume of corn seedlings, but the promotion effect was not significant as the proportion of cover crops increased. The root volume of corn under the four treatments of ryegrass increased significantly compared with CK. All the WC treatments significantly improved the root activity of corn, and the 3:100 SSR treatment recorded the highest root activity. The root activity significantly increased when the SSR of the RG treatment was lower than 10:100, while the activity was significantly inhibited when the SSR reached 10:100. Compared with WC and RG treated with 10:100 SSR, RG inhibited corn roots; however, root indicators of corn under WC treatment were still promoted (Table 3).

Depending on the morphological indicators, we can conclude that cover crops returning to the field had a "hormesis effect" on corn, and the effect of RG was more obvious. All SSRs of WC improved the plant height of corn, while 1:100 and 3:100 SSRs of RG improved the corn height but significantly inhibited the corn height when the SSR exceeded 3:100 (Figure 6A). The corn stem diameter was slightly improved as the SSR of WC and RG was within 3:100. The stem diameter of corn was significantly inhibited at 10:100 SSR (Figure 6B). The fresh weight of corn was significantly improved when the SSR of WC was within 6:100 and RG was within 3:100 (Figure 6C).

Table 3. Effect of different soil and straw ratios on the corn root system [mean (SD); *n* = 5].

Treatment	Total Length (cm)	Average Diameter (mm)	Surface Area (cm^2)	Volume (cm^3)	Branch Points	Root Tips	Root Activity ($\mu g \cdot g^{-1}$)
CK	695.51 (176.11) b	6.70 (0.34) b	131.43 (19.37) c	6.03 (1.66) b	3827.00 (621.30) c	5081.60 (441.25) c	1.90 (0.011) e
WC 1:100	977.47 (145.46) a	8.00 (0.26) a	258.18 (27.39) a	12.84 (2.10) a	5976.25 (733.56) b	8273.40 (945.04) a	3.30 (0.0080) b
WC 3:100	1092.56 (45.51) a	8.10 (0.29) a	283.08 (15.28) a	13.03 (3.24) a	6605.00 (334.08) b	6923.00 (1537.07) ab	6.00 (0.02) a
WC 6:100	1151.40 (117.28) a	5.80 (0.058) c	205.67 (21.49) b	7.28 (1.26) b	7503.40 (749.19) a	6895.40 (1248.09) ab	2.20 (0.01) d
WC 10:100	1041 (116.41) a	6.90 (0.14) b	225.15 (27.07) b	8.69 (1.22) b	6249.80 (1325.17) b	6500.60 (1377.52) bc	2.70 (0.023) c
CK	695.51 (176.11) c	6.70 (0.34) a	131.43 (19.37) b	6.03 (1.66) c	3827.00 (621.30) c	5081.60 (441.25) b	1.90 (0.011) d
RG 1:100	938.85 (92.10) b	6.60 (0.46) a	195.61 (12.64) a	8.33 (0.99) a	6105.00 (591.51) a	5519.00 (895.1) b	3.80 (0.021) a
RG 3:100	1140.94 (75.59) a	5.70 (0.060) b	204.50 (11.22) a	7.37 (1.19) ab	6917.80 (500.36) a	9357.50 (1723.64) a	2.60 (0.020) b
RG 6:100	1127.63 (114.68) a	5.90 (0.27) b	212.24 (20.74) a	8.85 (1.23) a	6724.25 (583.18) a	8793.75 (1913.43) a	2.50 (0.021) c
RG 10:100	974.30 (93.14) b	6.00 (0.29) b	196.96 (24.67) a	7.72 (1.39) ab	5215.00 (553.67) b	5792.25 (1141.54) b	1.60 (0.0080) e

1. WC, white clover; RG, ryegrass. 2. Numbers followed by different letters within each column were significantly different ($p < 0.05$) among different soil and straw ratios for the same treatment.

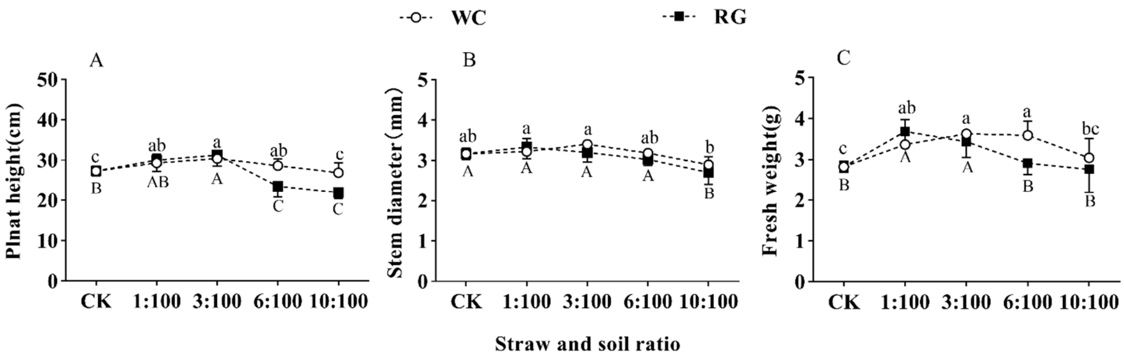

Figure 6. Straw returning on seedlings morphological properties ((**A**) Plant height,(**B**) Stem diameter, (**C**) Fresh weight). Different upper- and lower-case letters represent significant differences ($p < 0.05$) between treatments in RG and WC, respectively. The symbol represents mean values of 5 replicates ± SD.

3.4.2. Effect of Straw Return on Physiological Characteristics of Corn

1. Effect of straw return on protective enzyme activity and MDA content of corn.

In the WC treatment, SOD activity did not significantly improve at an SSR of 6:100, but it was significantly inhibited at the 10:100 SSR treatment (Figure 7A). The trends of POD, CAT activity, and MDA content were similar; all three were decreased at the 1:100 SSR treatment where the changes in CAT activity and MDA content were significant, significantly improved at the 3:100 SSR treatment, and then significantly decreased as the SSR exceeded 6:100. In the RG treatment (Figure 7B–D), SOD activity was significantly decreased in the 3:100 SSR treatment and significantly improved in the 10:100 SSR treatment (Figure 7E). The tendencies of POD and CAT activity were similar; both significantly declined in the 1:100 SSR treatment and improved in the 3:100 SSR treatment where the change in POD activity was significant, and then significantly decreased as the SSR exceeded 6:100 (Figure 7F,G). The MDA content in the RG treatment declined gradually with increasing SSR, and all the treatments reached a significant level (Figure 7H).

2. Effect of straw return on chlorophyll- and photosynthesis-related properties of corn.

The effect of the SSR of WC on the photosynthesis of corn showed that a low SSR (≤3:100) can improve all photosynthetic indices except the instant water use efficiency (iWUE), while an SSR ≥ 6:100 would inhibit some photosynthetic indices, such as the transpiration rate and intercellular carbon dioxide concentration; the inhibition phenomenon disappeared in the 10:100 SSR treatment. It is interesting to note that a high SSR (≥6:100) of WC can significantly improve the iWUE of corn. The 1:100 SSR of RG significantly improved the net photosynthesis, transpiration rate, and stomatal conductance of corn, while the high SSR (≥6:100) showed the opposite effect. The 3:100 and 10:100 SSR treatments recorded high iWUE values, and the highest intercellular carbon dioxide concentration appeared in the 6:100 treatment (Table 4). The effect of the low SSR treatment (≤3:100) of RG and WC on corn chlorophyll was not significant. When the SSR of WC reached 10:100 and the SSR of RG exceeded 6:100, the SPAD value significantly declined (Figure 8).

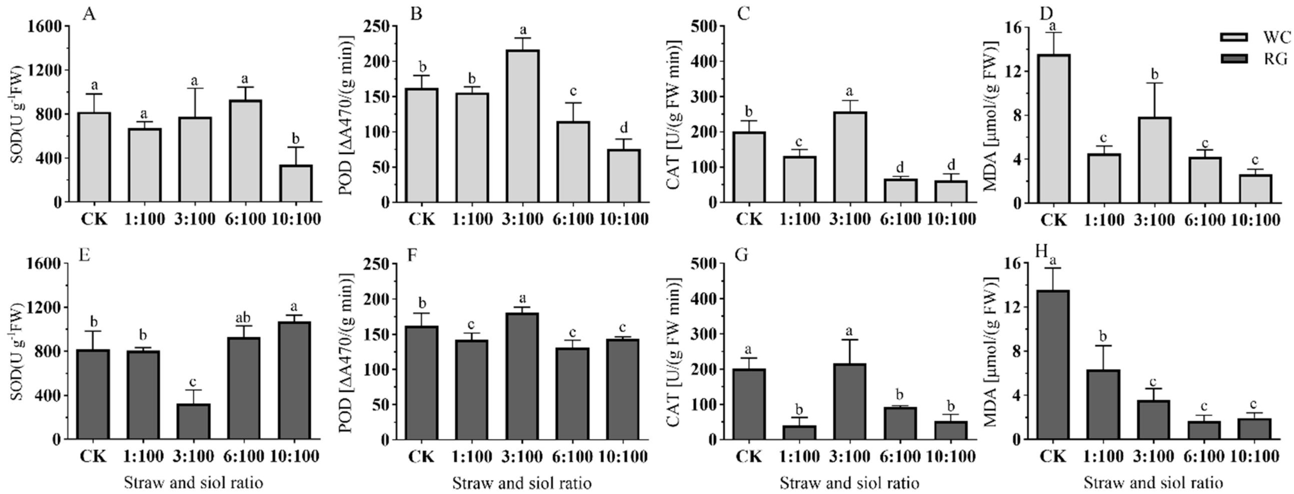

Figure 7. Effect of cover crop turnover on protective enzyme activity and MDA content of corn. (**A**–**D**) represent related enzyme activities and MDA content in white clover (WC) treatment; (**E**–**H**) represent related enzyme activities and MDA content in ryegrass (RG) treatment. Different lower-case letters represent significant differences (p < 0.05) between treatments in RG and WC. The bar represents mean values of 5 replicates ± SD.

Table 4. Effect of different soil and straw ratios of WC and RG on corn photosynthesis properties [mean (SD); n = 15].

Treatment	Pn (μmol·m^{-2}·s^{-1})	E (μmol·m^{-2}·s^{-1})	Gtc (μmol·m^{-2}·s^{-1})	Ci (μmol·mol^{-1})	iWUE (μmol·mmol^{-1})
CK	16.93 (3.63) d	1.48 (0.20) d	66.61 (10.13) c	125.88 (28.89) ab	11.37 (1.09) c
WC 1:100	20.6 (2.45) b	2.12 (0.11) b	88.37 (4.43) ab	142.11 (19.68) a	9.68 (0.79) d
WC 3:100	22.46 (1.92) a	2.28 (0.33) a	90.73 (14.76) a	126.30 (16.88) ab	9.96 (0.84) d
WC 6:100	18.82 (2.23) c	1.38 (0.20) d	69.26 (11.88) c	102.62 (25.20) c	13.73 (1.04) a
WC10:100	20.01 (2.16) bc	1.64 (0.30) c	82.88 (15.83) b	128.93 (39.4) ab	12.49 (1.87) b
CK	16.93 (3.63) b	1.48 (0.20) b	66.61 (10.13) b	125.88 (26.89) b	11.37 (1.09) b
RG 1:100	19.55 (2.30) a	1.81 (0.29) a	76.69 (9.89) a	119.98 (20.51) b	10.88 (0.68) b
RG 3:100	16.99 (2.97) b	1.41 (0.30) b	69.59 (17.06) b	130.31 (22.56) b	12.19 (0.94) a
RG 6:100	12.83 (0.66) c	1.15 (0.055) c	55.29 (2.80) c	149.40 (22.14) a	11.21 (1.03) b
RG10:100	12.77 (1.26) c	1.04 (0.14) c	50.41 (7.09) c	126.36 (32.54) b	12.42 (1.64) a

1. Net photosynthetic rate (Pn); transpiration rate (E); stomatal conductance (gtc); intercellular carbon dioxide concentration (Ci); instant water use efficiency (iWUE). 2. WC, white clover; RG, ryegrass. Numbers followed by different letters within each column were significantly different (p < 0.05) among different soil and straw ratios for the same treatment.

The 1:100 SSR of WC had no significant effect on the fluorescence indicators of corn except that NPQ. 3:100 and 6:100 SSRs improved all the fluorescence indicators of corn except Fv/Fm, among which the qP value reached a significant level compared with CK. When the SSR of WC exceeded 10:100, all the fluorescence indicators except qP and NPQ declined, and φPSII and ETR reached significant levels. The effect of RG straw turnover on corn was insignificant at low SSRs but significantly decreased the fluorescence indicators at high SSRs except for NPQ. The NPQ values of WC and RG gradually improved as the SSR increased, and all the variations compared to CK were significant (Table 5).

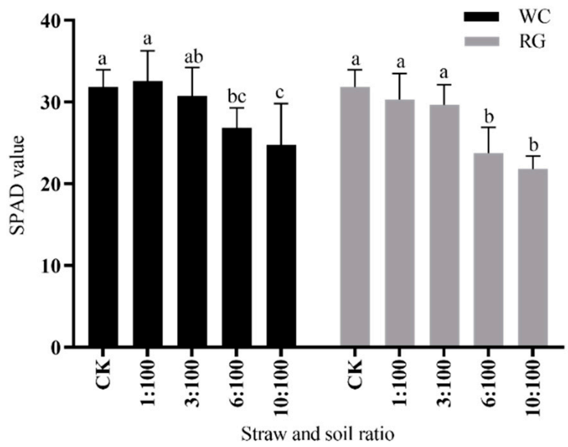

Figure 8. Effect of cover crops turnover on SPAD value of corn. Different lower-case letters represent significant differences ($p < 0.05$) between treatments in RG and WC. The bar represents mean values of 5 replicates ± SD.

Table 5. Effect of different soil and straw ratio of WC and RG on corn fluorescence properties [mean ± (SD); $n = 15$].

Treatment	Fv/Fm	φ PSII	ETR (μmol·m^{-2}·s^{-1})	qP	NPQ
CK	0.65 (0.027) a	0.21 (0.026) a	97.13 (11.93) a	0.52 (0.045) c	3.02 (0.35) c
WC 1:100	0.57 (0.039) c	0.21 (0.0021) a	94.91 (0.67) a	0.52 (0.050) c	3.58 (0.022) b
WC 3:100	0.59 (0.048) bc	0.23 (0.022) a	104.3 (10.06) a	0.59 (0.018) a	3.41 (0.13) bc
WC 6:100	0.57 (0.063) c	0.22 (0.0094) a	103.8 (4.36) a	0.60 (0.021) a	3.39 (0.36) bc
WC10:100	0.61 (0.031) b	0.19 (0.019) b	86.89 (8.99) b	0.55 (0.033) b	5.01 (0.82) a
CK	0.65 (0.027) a	0.21 (0.026) a	97.13 (11.93) a	0.52 (0.045) ab	3.02 (0.35) d
RG 1:100	0.67 (0.036) a	0.21 (0.023) a	95.91 (10.59) a	0.54 (0.027) a	3.63 (0.20) c
RG 3:100	0.61 (0.056) b	0.22 (0.016) a	99.94 (7.52) a	0.55 (0.034) a	4.01 (0.24) b
RG 6:100	0.58 (0.043) c	0.19 (0.040) b	85.57 (18.36) b	0.52 (0.052) ab	4.15 (0.43) b
RG10:100	0.61 (0.026) bc	0.17 (0.025) b	77.47 (11.50) b	0.50 (0.037) c	4.76 (0.43) a

1. Maximal quantum yield of PSII (Fv/Fm); PSII actual photochemical quantum efficiency (φPSII); electron transport rate (ETR); photochemical quenching (qP); and nonphotochemical quenching (NPQ). 2. Numbers followed by different letters within each column were significantly different ($p < 0.05$) among different soil and straw ratios for the same treatment.

4. Discussion

Rotation tillage and cover-cropping applications are important parts of conservation tillage. Returning *Trifolium subterraneum* L. to the field as green manure enhanced soil fertility, and reduced the weed seed bank size [23,24]. The use of ryegrass as a cover crop at sparse planting density can improve rice yield and suppress weed growth in the paddy field [25]. Through two years of field experiments and laboratory experiments, the results showed that different cover crops and tillage systems significantly influenced corn yield and weed infestation. NT recorded the highest cover crop biomass and less weed infraction, but the corn yield in NT was the lowest compared to the three cover crop treatments in RT. Previous studies have found that straw mulching combined with NT treatment can improve crop grain yield [26]. In our view, the direct reason that NT affects crop yields is the type of soil and the type of crop. Planting dryland crops on NT clay can lead to lower yields. In RT treatment, the WC system recorded the highest total corn yield (20.20 t ha^{-1}) among six treatments, followed by the FL system (18.46 t ha^{-1}) and the RG system (17.86 t ha^{-1}). Cover crops efficacy is influenced by root function and the presence of plant straw on soil surfaces [4]. Root covering and straw mulch does provide weed suppression, but the dense layer of aerial roots was formed in the RG field, which led to soil compaction, and might be one of the reasons why the lowest corn yield was recorded in the RG treatment. The second reason for the harmful effects on corn was the phytotoxic potential of RG. According to the pot experiment, a large amount of RG biomass returned to the soil would cause emaciated morphological characteristics of corn, including plant height, stem diameter, and fresh weight. Moreover, the decline in the SPAD value hinted that the chlorophyll content was reduced, which was associated with a decline in photosynthetic indicators and fluorescence indicators. The change in protective enzyme activity of corn under different SSR treatments was nonlinear. Researchers found that different proportions of corn straw returned to the soil can affect the community composition of soil microbes, but the signatures (PLFAs) were nonlinear with straw and soil ratios [27]. Soil flora affects the decomposition rate of plant stubble and the release concentration of allelopathic compounds, which may be associated with the nonlinear change in protective enzyme activity in our study. The activities of POD and CAT under the 3:100 SSR treatment of WC and RG were improved, and then the activity significantly declined as the SSR increased. Combining the morphological and physiological parameters, these enzyme changes were within the appropriate range of variation, which means that corn was not harmed under those SSR conditions. This result was similar to the effect of cotton (*Gossypium* spp.) extract on the growth of several crops [28]. The SOD activity significantly improved at 10:100 SSR in the RG treatment, which hinted that the corn might be at risk from superoxide. Overall, according to the MDA content, WC and RG returning to the field did not lead to peroxidation of the corn lipid membrane at the ratio of straw and soil set in the experiment. We found that different SSRs of WC and RG have no significant side effects on the corn root system even in the 10:100 SSR treatment. However, corn roots grow better at low SSRs, and depending on the variation in root growth at different SSRs, exceeding the SSR set in the experiment may be detrimental to the root growth of corn. A study found that *Ludwigia hyssopifolia* (G. Don) aqueous extract can cause root reduction in rice (*Oryza sativa* L.), and the effect of the extract on rice growth parameters was concentration-dependent [17], which is consistent with the findings of this study. Oregano (*Origanum vulgare* L.) biotypes incorporated with green manure can improve cotton yield, and the weed inhibition ability is also excellent. The author claimed that a high phenolic content of oregano biotypes was feasible to suppress weeds [29]. In this study, although no screening of different green manure biotypes was carried out, the relatively higher phytotoxic release concentrations of RG straw in the surface soil of the NT field compared to the RT field may have contributed to the reduced corn yield. Researchers found that the combined application of fertilizer and milk vetch can effectively improve the content of chlorophyll and the net photosynthesis rate of rice, which enriched crop yield [30]. The reason why planting WC had no significant effect on the yield of corn was that the phytotoxic potential of WC had no negative effect on corn growth. On the contrary, the participation of WC can significantly improve the morphological and the physiological properties of corn; even though the mean fresh weight of WC biomass reached 32.67 t ha^{-1} in the second year, there were no side effects on the growth and yield of corn. On the contrary, a high proportion of RG residue turnover into the field is harmful to the photosynthesis-related indicators as well as chlorophyll content, which may be the main cause of corn crop failure. The laboratory experimental results were consistent with the field trial results.

The normalized difference vegetation index (NDVI) can be used as a source of information for improving the accuracy and precision of cover crop biomass prediction [31]. In this study, RG showed the fastest growth in the early stage compared to the natural weeds and WC. WC gradually formed a population after two months of sowing. The cover crop population in the WC field includes many natural weed species, which indicates the weak weed inhibition ability of WC. However, the biomass of WC in the second year was quite significantly higher than that in the other cover crop treatments because the cold resistance ability of WC was strongest. The total weed biomass of the first and second years was RT-WC > RT-FL > NT-FL > NT-WC > RT-RG > NT-RG, which can be due to the influence of tillage methods and cover crop adoption. Researchers have found that cover crops have different effects on weed management, which depend on the use of straw [32]. In this study, RG showed the highest total weed biomass and different species of weed inhibition ability under the different tillage practices. However, WC can inhibit or improve certain kinds of weed species under certain conditions. The effects of different tillage methods and different cover crops are delayed. Compared to the first year, corn yield improved, and the weed biomass in all the treatments declined in the second year, which showed good efficacy. Tillage management is the main factor in weed infractions. Soil disturbance, i.e., bed-sown, conventional tillage and minimal tillage, etc. can resist weed infestation [33–35]. It was difficult to conduct efficient weed management manually in NT fields, especially fallow fields in winter. In the second year, many natural weeds will grow on fallow land, which can cause problems for farming. Burning straw is inhibited in China, so people normally use nonselective herbicides, but the straw of fibrous weeds, such as goosegrass, will affect the farming operation of rotary cultivators. The cover crops in this study are annual, and they will stop growing and even die as the temperature rises in late spring. The characteristics of heat death are more evident on RG, which can reduce the use of herbicides. Weed green control occurs in a variety of ways; the use of cover crops alone [36,37], or as combined with mechanical operation [38], nutrient management [39], effective herbicide [40], or even putting loach in the paddy fields [41], can significantly inhibit weed growth. In this study, planting RG in fallow fields and using a mower to return the aboveground part to the field before planting corn was highly suggested. Researchers found that long-term milk vetch return to rice paddies in milk vetch-rice rotation systems can significantly decrease the number of seed banks but increase the species of weeds, which can balance the ecosystem of rice paddies [42]. Organic farming relies on the correct use of farmyard manure, compost, vermicompost, green manures, and several other factors [43]. Cover crops returning to the field are organic sources for the amelioration of soil physical, chemical, and biological properties [44]. Furthermore, green manure conserves the soil available nutrients [45] and suppresses the losses incurred due to erosion, leaching, etc. [3]. Not only that, planting green manure can inhibit several diseases, pests, and weeds [43]. In our study, NT inhibited weed infraction and was a combined factor, that included the competitive ability of RG at an early stage after sowing, high vegetation coverage, coverage of the aerial root, and the release of phytotoxicity. Moreover, the return of a certain amount of cover crops gives the corn a significant advantage, which can improve its ability to compete with weeds.

5. Conclusions

RG combined with RT can reduce weed infestation while ensuring a relatively high corn yield. FL was not an appropriate choice because of the most serious weed invasion. RT-WC had the highest weed incidence, followed by RT-FL, and NT-RG had the lowest weed incidence. NT was not suitable for corn-based crop rotation systems because of its low corn yield. RT-WC recorded the highest corn yield over two years, while NT-RG recorded the lowest corn yield. The weed-inhibiting potential of RG was better than that of WC, which can contribute to the field cover capacity and phytotoxic potential. High SSR ($\geq$10:100) of RG straw returning to the field also has a significant phytotoxic effect on the growth of corn. A high proportion of WC straw returning to the field had no significant effect on the growth of corn because the phytotoxic potential of WC was relatively weak compared to RG. It was feasible to practice RT combined with an RG-corn rotation system in South China, which could be a feasible and environmentally friendly tool for weed control in organic farming systems. However, the amount of RG returned to the field needs to be controlled to prevent negative impacts on corn growth. WC application combined with multiple weed management practices was necessary such as spraying pre-emergence herbicide (i.e., s-metolachlor, Dual Gold®) after soil tillage to prevent weed infestation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12050999/s1, Figure S1: The plots distribution.

Author Contributions: Conceptualization, S.L. and Y.M.; experimental method and design, software and original draft and writing, S.L.; data curation, Y.Z.; Z.C. and X.D. revised the paper; funding acquisition, supervision and validation, Y.M. and Z.M. All authors have read and agreed to the published version of the manuscript.

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