Tillage and irrigation increase wheat root systems at deep soil layer and grain yields in lime concretion black soil

Jinfeng Wang¹,², Zhuangzhuang Wang¹,², Fengxu Gu¹,², Huan Liu¹,², Guozhang Kang¹,²,³, Wei Feng¹,²,³, Yonghua Wang¹,²,³, & Tiancai Guo¹,²,³

In lime concretion black soil, a two-factor (tillage and irrigation) split block experiment from 2015 to 2017 was conducted to identify whether their combination is suitable for the improvement of winter wheat yield and water use efficiency. The main treatments were subsoiling (SS) and rotary tillage (RT), with secondary treatments of three irrigation regimes: no irrigation during the whole growth period (W0), irrigation at jointing stage (W1), and irrigation at both jointing and anthesis stages (W2). In combination with a soil column experiment, the contribution of the root system in different soil layers to yield was clarified. The results indicated that both tillage and irrigation significantly influenced the spatiotemporal distributions of the root systems and yield components, while tillage produced the strongest effect. Compared with RT, SS significantly promoted the root penetration and delayed root senescence in deep soil layers. With increasing soil depth, each root configuration parameter (dry root weight density, DRWD; root length density, RLD; root surface area per unit area, RSA; root volume per unit area, RV) gradually decreased, and the peak appearance times of each root parameter in RT and three parameters (RLD, RSA and RV) in SS were postponed from heading to anthesis and from anthesis to filling stage, respectively. The average post-peak attenuation values at soil layers from 60 to 100 cm in W1 were less than those in W0 and W2. SSW1 generated the highest grain yields, with an average increase of 31.88% compared with the yield in RTW0. Root systems at three soil layers (0–40 cm, 40–80 cm and below 80 cm) differentially contributed to grain yields with 78.32%, 12.09% and 9.59%, respectively. The growth peak of the deep root system in SSW1 was postponed to the filling stage, and the post-peak attenuation declining rates were also slowed. Therefore, SSW1 is an effective cultivation method improving grain yields and water use efficiency in lime concretion black soil.

Wheat (Triticum aestivum L.), one of the most important food crops in the world, is the main source of daily protein and provides 20% calories consumed by humans¹. China has the largest wheat growth areas and productions with 2.3 × 10⁷ ha and 1.3 × 10⁸ t in 2020, respectively. There are about 3.2 × 10⁶ ha lime concretion black soil in China, and it accounts for 13.9% of wheat growth areas and is mainly distributed in Anhui, Henan, Shandong and Jiangsu provinces. In this soil type, wheat grain yields are lower over 15% than those in other types of soil, because its groundwater is shallow, and water shortages often occur under dry weather conditions, especially in growth seasons with strong evaporation and high water consumption². During these water shortage periods, the rise of capillary water hardly keeps up with the loss due to evaporation and transpiration in the upper layer of the soil. Thus, the supplementary irrigation is necessary to ensure a high and stable yield. In recent years, successive RT, heavy machinery rolling, and unsuitable land preparation operations have caused changes in the soil structure of most wheat fields, especially those with lime concretion black soil³,⁴. With such operations, the plough layer becomes shallower, the plough pan is thickened and moved upward, soil compaction and bulk density are increased, and the transduction conditions of water, vapour and heat are destroyed⁵. Moreover, soil compaction stress and water conduction obstruction have become important issues affecting wheat root growth,
yield formation and the improvement of water and fertilizer utilization efficiency. In addition, the conditions of field irrigation facilities in this region are relatively bad, and most of farmers neglect irrigation. In lime concretion black soil, therefore, agronomic measures such as tillage and irrigation can effectively improve the physical properties, reduce the imbalance between the soil water storage and supply, and create a soil environment conducive to wheat root growth and high efficient water utilization to achieve simultaneous improvement in wheat grain yield and water use efficiency. These are key measurements to improve the comprehensive production performance of winter wheat in this region.

In lime concretion black soil, the composition, structure and porosity of lime concretion black soil are very different from other soils, which cause this soil to have a low water-holding capacity, weak hydraulic conductivity, slow capillary water rising speed and a small rising height. Lime concretion black soil is characterized with undesirable properties, such as high viscosity, dry shrinkage, wet expansion, poor soil structure and short suitable tillage time, which have become the main factors limiting further increases in winter wheat yield.

Previous studies have shown that compared with traditional cultivation, optimized cultivation improves the wheat root distribution, delays wheat root senescence, and promotes the absorption of water and nitrogen in deep soil, all of which increase wheat production. Strip RT after SS is a high-yield, efficient and water-saving tillage practice that can increase farmland water consumption, enhance stored soil water consumption, promote dry matter accumulation and increase the photosynthetic rate and water use efficiency of flag leaves. In addition, once or twice suitable irrigations are beneficial to obtain higher yield and water use efficiency, and the irrigation water use efficiency is highest when irrigation is operated at the critical period of water. The CERES-Wheat model can simulate and predict the effects of different irrigation modes. Studies have shown that the application of 75 mm of irrigation water during jointing and anthesis is the best irrigation strategy in the North China Plain, and it is believed that one irrigation event at the jointing stage can be used as an alternative irrigation mode to reduce irrigation and maintain the sustainable development of farmland water resources. In semi-arid areas, the ridge-furrow rainfall harvesting system combined with 75 mm of irrigation increases soil moisture across the rooting area, making it a high-yield and efficient water-saving strategy.

In previous studies, the effects of single factor, such as variety, planting density, cultivation pattern, fertilizer application, irrigation amount, and irrigation frequency, on root growth and wheat development were examined. The researches about irrigation–tillage mostly focused on soil properties, greenhouse gas emissions, water use efficiency and yield. Gajri and his colleagues showed that deep tillage and early irrigation could shorten the time needed for root to reach a specified depth, thus improving water use efficiency and achieving high yield. For lime concretion black soil, it has been shown that excessive irrigation is not conducive to the efficient use of water and the combination of SS and one irrigation event at jointing is a suitable cultivation pattern that results in simultaneous increases in winter wheat yield and water use efficiency in this region. However, the regulatory effects of tillage and irrigation on the root parameters of winter wheat remain unclear in different soil layers in lime concretion black soil. In this study, tillage, irrigation, and their interaction were implemented in the field conditions of the lime concretion black soil for two wheat growth seasons. To evaluate the contribution of roots in different soil layers to grain yields, in addition, soil column experiment was also conducted. The objective of this study was to explore the effects of tillage and irrigation on root characteristics and grain yields in lime concretion black soil, and clarify their regulatory mechanisms underlying the suitable wheat root construction in this soil type.

Results

Effects of tillage and irrigation on yield. As shown in Table 1, the significant effects of tillage and irrigation on grain yield and its components during the wheat growing season were similar between the two years. Grain yield and its components in SS were higher than those in the RT treatment, except the kernel number per spike in 2016–2017. However, in both tillage treatments, the yield in W1 was the highest, and that in W0 was the lowest. The average yield in W1 was 22.18% and 24.16% higher than that in W0 under SS and RT, respectively. Moreover, the yield in SSW1 was the highest in the two years, with an average increase of 31.88% compared with the lowest value, which was observed in treatment RTW0. Under a given tillage method, with an increase in the number of irrigation events, spike number showed an increasing trend, thousand-grain weight showed a decreasing trend, and the regularity of kernel number per spike varied. However, while there was no significant difference between W0 and W1 in the first year of the experiment, the kernel number per spike under both the RT and SS conditions reached a maximum value in the W1 treatment in the two years. In 2016–2017, the average kernel number per spike in W1 increased by 21.08% and 8.63%, respectively, compared with that in W0 and W2.

According to the results of variance analysis, the effects of different combination treatments on thousand-grain weight and grain yield in the two-year test were obvious, while the effects on spike number and kernel number per spike were unstable. The tillage and irrigation obviously affected grain yields mainly by regulating the thousand-grain weight, and the spike number and thousand-grain weight, respectively. However, the interaction effects of tillage and irrigation were insignificant.

Effects of tillage and irrigation on root development indices and root architecture. The effects of tillage or irrigation on root parameters was significant and in different degree; however, the interaction effect of tillage and irrigation was not significant, and the main regulatory effect was of tillage (Tables 2 and 3). The total dry root weight (TDRW), total root length (TRL), total root surface area (TRSA) and total root volume (TRV) in the SS treatment in each growth period were all higher than those in the RT treatment. Compared with RT, TDRW, TRL, TRSA and TRV parameters in SS increased by 3.52–47.56%, 7.16–46.38%, 6.48–53.14%, and 6.22–123.80% (2015–2016), and 6.54–34.38%, 5.16–54.87%, 12.54–49.09%, and 8.52–63.88% (2016–2017), respectively. Moreover, during the two-year test, the TDRW, TRL, TRSA and TRV in W1 were better than those
in the other irrigation treatments. In comparison to W0, the above parameters in W1 increased by 1.06–53.78%, 3.33–29.42%, 4.78–60.44%, and 3.27–75.52% (2015–2016), respectively. And similar results appeared in 2016–2017 wheat growth season (Tables 2 and 3). Compared with W2, similar increasing proportions of the above parameters in W1 appeared with 1.71–12.02% and 1.20–8.22%, 1.12–11.02% and 1.33–15.15%, 2.01–11.33% and 0.92–10.57%, and 1.38–9.90% and 1.79–14.01% in two wheat growth seasons (2015–2016 and 2016–2017), respectively.

Table 1. Effects of tillage practice and irrigation regime on the yield and yield components of winter wheat in lime concretion black soil. Data followed by different lowercase letters within any column indicate that the difference is significant at the P = 0.05 level. *, **, and *** indicate significant differences at the P = 0.05, P = 0.01 and P = 0.001 levels, respectively. The same as below.

| Years | Treatments | Spike number (10^4·ha⁻¹) | Kernels per spike (kernel·spike⁻¹) | Thousand-grain weight (g) | Yield (kg·ha⁻¹) |
|-------|------------|---------------------------|-----------------------------------|--------------------------|----------------|
| 2015–2016 | SS | W0 | 536.18ab | 38.92ab | 46.35a | 6984.60d |
|        |        | W1 | 569.49a  | 40.48a   | 43.56bc | 8507.85a |
|        |        | W2 | 573.53a  | 37.59bc  | 42.07 ed | 7833.30b |
|        | RT | W0 | 497.40b  | 38.70ab  | 41.69 ed | 6528.60e |
|        |        | W1 | 536.77ab | 37.45bc  | 40.51d  | 8046.90b |
|        |        | W2 | 534.39ab | 35.60c   | 40.05d  | 7563.15c |
|        |      | Tillage | 5.52*    | 8.93*    | 29.83** | 149.69*** |
|        |      | Irrigation | 2.44     | 6.88*    | 129.27c |
|        |      | Tillage*Irrigation | 0.02 | 1.97 | 0.30 | 0.55 |
| 2016–2017 | SS | W0 | 683.98bc | 29.43c  | 44.94a | 8129.27c |
|        |        | W1 | 732.83ab | 36.67a   | 41.02bc | 9277.32b |
|        |        | W2 | 770.82a  | 33.50b   | 40.01 ed | 7833.30b |
|        | RT | W0 | 609.88c  | 30.87c   | 42.74ab | 7474.23d |
|        |        | W1 | 717.78abc | 36.34a | 39.97 cd | 9393.25b |
|        |        | W2 | 726.12ab | 33.71b   | 38.22d  | 8407.08c |
|        |      | Tillage | 3.22     | 0.43     | 6.59** | 56.40*** |
|        |      | Irrigation | 8.46**   | 29.97*** | 119.17a |
|        |      | Tillage*Irrigation | 0.32    | 0.61    | 0.18   | 0.68 |

Table 2. Effects of tillage practice and irrigation regime on the total dry root weight and total root length of winter wheat.

| Year | Treatment | Total dry root weight (TDRW)/g·m⁻² | Total root length (TRL)/cm·cm⁻² |
|------|-----------|-----------------------------------|-------------------------------|
| 2015–2016 | SSW0 | 49.55a | 94.76c |
|        | SSW1 | 49.30a | 133.77a |
|        | SSW2 | 48.50a | 130.11ab |
|        | RTW0 | 41.74b | 85.19b |
|        | RTW1 | 42.96b | 133.85a |
|        | RTW2 | 42.12b | 127.43b |
|        | Tillage practice | 58.12*** | 9.97** |
|        | Irrigation regime | 0.26 | 463.73*** |
|        | T*I | 0.31 | 4.98* |

| Year | Treatment | Total dry root weight (TDRW)/g·m⁻² | Total root length (TRL)/cm·cm⁻² |
|------|-----------|-----------------------------------|-------------------------------|
| 2016–2017 | SSW0 | 75.81b | 110.50c |
|        | SSW1 | 78.52a | 132.49a |
|        | SSW2 | 78.14a | 125.77b |
|        | RTW0 | 70.05d | 100.55f. |
|        | RTW1 | 74.80bc | 119.57c |
|        | RTW2 | 73.35c | 113.88d |
|        | Tillage practice | 65.63*** | 344.14*** |
|        | Irrigation regime | 14.62*** | 377.81*** |
|        | T*I | 1.00 | 1.95 |

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### Table 3. Effects of tillage practice and irrigation regime on the total root surface area and total root volume of winter wheat.

| Year | Treatment | Total root surface area (TRSA) × 10^3 m^2·ha^−1 | Total root volume (TRV)/m^3·ha^−1 |
|------|-----------|----------------------------------------|----------------------------------|
|      | Jointing  | Heading | Anthesis | Filling | Maturity | Jointing | Heading | Anthesis | Filling | Maturity |
| 2015–2016 | SSW0 | 196.49 | 490.33 | 576.39 | 410.18 | 275.59 | 78.47 | 142.74 | 216.82 | 151.82 | 92.08 |
|      | SSW1 | 204.29 | 583.34 | 681.95 | 634.77 | 489.39 | 81.67 | 185.52 | 254.10 | 223.84 | 172.63 |
|      | SSW2 | 201.47 | 556.47 | 644.03 | 592.47 | 440.85 | 80.55 | 179.14 | 223.84 | 172.63 | 161.56 |
|      | RTW0 | 183.30 | 419.16 | 406.78 | 288.32 | 220.46 | 73.46 | 138.20 | 132.35 | 69.76 | 49.62 |
|      | RTW1 | 193.66 | 551.30 | 512.47 | 440.85 | 81.67 | 104.98 | 216.28 | 163.43 | 97.74 | 64.77 |
|      | RTW2 | 188.64 | 506.76 | 494.11 | 382.27 | 274.00 | 74.21 | 167.19 | 163.43 | 69.76 | 49.62 |
|      | Tillage practice | 30.31*** | 5.62* | 104.58*** | 179.16*** | 811.16*** | 593.28*** | 460.97*** | 92.84*** | 674.00*** | 1689.73*** |
|      | Irrigation regime | 5.62* | 246.76*** | 179.16*** | 811.16*** | 593.28*** | 460.97*** | 92.84*** | 674.00*** | 1689.73*** |
| 2016–2017 | SSW0 | 319.66 | 596.39 | 675.64 | 508.28 | 101.30 | 176.42 | 244.24 | 141.51 | 92.54 |
|      | SSW1 | 323.78 | 704.16 | 756.40 | 666.75 | 508.11 | 104.98 | 216.19 | 163.43 | 69.76 | 49.62 |
|      | SSW2 | 321.55 | 679.16 | 759.65 | 608.70 | 442.01 | 103.64 | 254.10 | 200.86 | 151.54 | 129.43 |
|      | RTW0 | 279.49 | 506.44 | 414.78 | 312.74 | 276.74 | 93.80 | 165.59 | 166.80 | 115.74 | 85.71 |
|      | RTW1 | 290.68 | 615.46 | 558.45 | 464.33 | 329.74 | 96.73 | 193.25 | 200.68 | 154.85 | 129.43 |
|      | RTW2 | 287.32 | 599.97 | 516.77 | 419.30 | 315.76 | 94.80 | 176.83 | 180.00 | 138.28 | 110.54 |
|      | Tillage practice | 629.27*** | 2.36 | 1044.13*** | 1196.11*** | 1523.31*** | 1347.90*** | 139.38*** | 88.41*** | 2343.77*** |
|      | Irrigation regime | 9.81** | 129.48** | 91.66** | 259.33** | 372.10** | 7.73** | 79.99** | 433.25** | 559.35** | 2754.79** |
|      | T1 | 2.36 | 1.52 | 6.68** | 0.44 | 91.74*** | 0.21 | 5.40** | 117.95*** | 106.63*** | 384.49*** |

### During the whole growth period, the root parameters under different combination treatments showed an inverted "V" trend, first increasing and then decreasing in both years. All root parameters in the SS treatment reached a peak at the anthesis stage, while those in the RT treatment mostly reached a peak earlier, at the heading stage. Moreover, four root configuration parameters (TDRW, TRL, TRSA and TRV) were the highest and lowest in the SSW1 and RTW0 treatments, respectively.

The figures (Figs. 1; 2; 3; and 4) show that the distributions of root growth characteristic parameters in different soil layers were different, and in the same growth period, the DRWD, RLD, RSA, and RV of different combination treatments gradually decreased as the soil depth increased. Considering the whole growth season, the DRWD, RLD, RSA, and RV in different soil layers for each combination treatment exhibited a single peak curve, rising first and then falling.

As soil depth increasing, the root parameters varied. In the upper soil layers (0–40 cm), the DRWD, RLD, RSA and RV under the RT treatment all reached their maximum value at the heading stage, while the maximum values under SS were postponed to the anthesis stage. In the middle soil layers (40–60 cm), the maximum values of DRWD and RV under RT reached at the heading stage and anthesis stage, respectively. The maximum values of RLD and RSA under RT were different in two years, which both appeared at the anthesis stage in 2015–2016 and the heading stage in 2016–2017. However, the peak appearance time of each root parameter under the SS treatment appeared at the anthesis stage. The change in root parameters below the 60 cm soil layers varied depending on the tillage method and growth year. In 2015–2016, the root growth parameters under RT peaked at the anthesis stage, except that of DRWD treated with RTW2, which appeared at heading. The DRWD under SS reached its maximum value at the anthesis stage, and the maximum values of RLD, RSA, and RV mostly appeared at the filling stage. However, in 2016–2017, the root parameter peaks in each combination treatment appeared at the anthesis stage. As soil depth increased and the growth period advanced, the peak time of root configuration growth parameters treated with RT tended to be delayed, that is, from the heading stage to the anthesis stage. However, the peak times of RLD, RSA and RV under SS were further delayed to the filling stage.

The effects of tillage and irrigation on root parameters in the middle and upper soil layers were not obvious, but the regulation of roots in the soil below 60 cm was significant. In the deep soil below 60 cm, the post-peak attenuation values of all root parameters in W1 decreased by 22.57% and 7.85% in comparison with W0 and W2, respectively. Compared with RT, the attenuation values under SS were reduced by 23.79% on average. Moreover, the post-peak attenuation values of DRWD, RLD, RSA, and RV in SSW1 were significantly lower than those in other treatments, and especially, they were lower by 31.65%, 40.17%, 44.23% and 50.35% than RTW0, respectively, in which the attenuation decreasing rates were highest.

**Effects of root cutting in different soil layer on yield.** Root cutting in different soil layers (no root cutting, CK; at 40 cm below the surface, T-40; at 80 cm below the surface, T-80) had a significant regulatory effect on winter wheat yield, with the T-40 treatment having the strongest regulatory effect (Table 4). The contribution rates of roots in the 0–40 cm, 40–80 cm and below 80 cm soil layers to yield were 78.32%, 12.09% and 9.59%, respectively. Root cutting in different soil layers reduced yield, and compared with that in CK, the grain yield in T-40 and T-80 decreased by 21.68% and 9.59%, respectively. Between T-40 and T-80, the significant differences...
in kernel number per spike and grain weight were caused by 40–80 cm root system, and between CK and T-80, the significant difference in grain weight was mainly due to the root below 80 cm (Table 4). Thus, the root sys-

Figure 1. Effects of tillage practice and irrigation regime on the dry root weight density of winter wheat in different soil layers. Note: a, b, c, d and e represent the soil layer of 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm and 80–100 cm, respectively.
tem in the 40–80 cm soil layer regulated the kernel number per spike and grain weight of wheat, while the roots below 80 cm mainly regulated grain weight to affect the yield.

Figure 2. Effects of tillage practice and irrigation regime on the root length density of winter wheat in different soil layers.
The relationships among root parameters, grain yield, soil water content, soil water use efficiency and precipitation water use efficiency. According to the correlation analyses between root growth parameters and grain yield (see Supplementary Fig. S1 online), the growth parameters TDRW, TRL, TRSA and TRV were positively correlated with grain yield to different degrees during each growth period of 2015-2016 and 2016-2017.

Figure 3. Effects of tillage practice and irrigation regime on the root surface area per unit area of winter wheat in different soil layers.
winter wheat. For TDRW and TRL, the correlation coefficient in the middle and late growth periods was relatively large, while for TRSA and TRV, there was little difference during the growth period except at the heading stage. All the correlation coefficients reached a maximum at the heading stage. In addition, in terms of their correlations with grain yield, the root parameters at the early growth stage ranked as TRV > TRSA > TRL > TDRW,
while at the late growth stage, they ranked as TDRW > TRL > TRSA > TRV. In addition, the above mentioned four root parameters were positively and significantly correlated with soil water content, soil water use efficiency and precipitation water use efficiency except for the relationship between TDRW and soil water content (see Supplementary Table S1 online).

Table 4. Effects of cutting roots in different soil layers on the yield and yield components of winter wheat.

| Treatment | Spike number (number/column) | Kernel per spike (kernel/spike) | Thousand-grain weight (g) | Yield (g/column) |
|-----------|------------------------------|---------------------------------|---------------------------|-----------------|
| CK        | 46.41a                       | 38.08a                          | 44.11a                    | 76.10a          |
| T-40      | 45.73a                       | 33.44b                          | 37.67c                    | 59.64c          |
| T-80      | 45.43a                       | 37.81a                          | 40.46b                    | 68.76b          |

Discussion

Effects of tillage and irrigation on the root spatiotemporal distribution. SS reduces soil compaction, increases DRWD and promotes root growth. This study found that the promotion effects of tillage on root structure were embodied in the following aspects: the root parameter peak in the RT treatment appeared earlier, and all the growth characteristic parameters in the 1 m soil layer reached a maximum at the heading stage, in contrast, SS delayed root senescence, and the peaks of TDRW, TRL, TRSA, and TRV were postponed to the anthesis stage. This may be due to the increased soil water consumption, precipitation water use efficiency and irrigation water use efficiency in SS treatment. Applying irrigation too early or too late during early growth is not conducive to root growth in the middle and late stages, and properly prolonging the irrigation date in the early growth stage can result in high yield and highly efficient root traits. Studies have shown that irrigation at the jointing and anthesis stages could increase the absorption area of deep roots. However, this study showed that the W1 irrigation mode slowed root senescence in the deep soil layer, because irrigation only at jointing can help roots penetrate the soil and increase the absorption and utilization of deep water. Excessive irrigation causes much ineffective evaportranspiration at the late growth stage, and thus water use efficiency greatly decreases. Furthermore, an appropriate irrigation deficit can enhance the soil enzyme activity in deep soil.

The influence of tillage under different soil layers on root configuration remain unclear. We found that, with increasing soil depth, the maximum appearance time of each root parameter was delayed overall. The parameter peak in the deep soil layer under RT was postponed to the anthesis stage, while that under SS was postponed to the filling stage, and the post-peak attenuation decrease was smaller than that in the RT treatment. This may because deep ploughing can reduce the soil bulk density of the 20–40 cm soil layer in lime concretion black soil and increase the soil porosity and field water-holding capacity of the 20–40 cm layer. And root growth parameters are determined by soil bulk density. These suggest that tillage methods can promote root growth by improving soil structure. Previous studies usually focused on the effects of single factor (tillage or irrigation) on root growth of winter wheat. However, there are few literatures about double factors and their interactions on root structures in different soil layers of winter wheat in lime concretion black soil. Ali and his colleagues found that, under the condition of 200 mm rainfall in a dry-land farming system, the ridge furrow rainfall harvesting technique with 150 mm deficit irrigation could significantly promote the physiological morphology of root system in the top 40 cm soil layer, resulting in higher grain yield. In another experiment, no-tillage promoted the accumulated density in shallow root system under drought condition and this is highly dependent on surface irrigation water. In this study, however, the regulation effects of tillage and irrigation on the root system were mainly manifested in the root system below 60 cm soil layer. The spatiotemporal distribution of root growth parameters in the 1 m soil layer under SSW1 was the best. Under this treatment, the peak appearance times of RLD, RSA and RV in deep soil layers were delayed. This study also indicated that under SSW1, the growth peak of the deep root system occurred later, and the post-peak attenuation decline was slowed. Thus, we consider a good root spatiotemporal distribution to be the main morphological or physiological factor causing high yield.

Effects of tillage and irrigation on grain yields. Xu et al. demonstrated that irrigation at anthesis and jointing with 150 mm of water was the best irrigation mode in the North China Plain. However, Meng et al. considered that irrigation applied at the wintering and jointing stages is good for nitrogen accumulation and utilization. If irrigation at anthesis was applied based on the pattern mentioned above, the proportion of accumulated nitrogen transferred to the grain would be significantly reduced, which would lead to decreases in nitrogen fertilizer utilization and yield. Similar results in single factor have previously been reported by Meng and his colleagues, indicating that the yield in the treatment of irrigation at jointing stage was the highest. However, two tillage treatments on the basis of single irrigation factor have been added in our study. Compared with RT, SS increases the transpiration rate, net photosynthetic rate and water potential of flag leaves. The results of our two-factor treatment showed that the yield in SSW1 treatment was highest in the lime concretion black soil of southeastern Henan. Zhang et al. reported that the TRSA, TRV and total number of root tips were significantly positively correlated with yield. In our study, root growth parameters were positively correlated with grain yields at different degrees, because there are positive correlations among root growth parameters, water use efficiency and nitrogen use efficiency. In addition, root parameters in our study were significantly and positively correlated with soil water content, soil water use efficiency and precipitation water use efficiency. Thus, it has been speculated that the root growth parameters could significantly affect the water and nitrogen absorption of winter wheat, then regulate the kernels per spike and the thousand-grain weight, and ultimately affect grain yield. In
Root contributions at different soil layers to grain yields. Guo et al.\textsuperscript{44} showed that the roots in soil layers above 20 cm and below 1 m contributed mostly to yield, especially the wheat plant roots below 1 m, which were more important for yield when wheat suffered drought at the late stage of growth. Moreover, Wang et al.\textsuperscript{45} showed that the root system of summer maize in the 0–40 cm soil layer had the greatest influence on nitrogen accumulation and transport after anthesis and thus had the greatest impact on yield. However, the root system in the soil layer below 80 cm had a significant influence on the thousand-grain weight of summer maize. This study revealed that the root system at 0–40 cm soil depth made the largest contribution to yield, but the roots at the lower and middle depths below 40 cm contributed as much as 21.68% to yield. Yield mainly depends on the shallow root system with abundant root biomass, but the deep root system has significant effects on the yield of crops such as wheat and rice\textsuperscript{46,47}. Therefore, the roots in deep layers play an important role in determining yield. For this reason, in production practices, promoting deep rooting and maintaining the quantity and activity of the deep root system should be key to achieving high and stable wheat yields.

Conclusions
In the lime concretion black soil, the root structures and distribution characteristics of winter wheat were positively related to soil water content, root water absorption characteristics and grain yields. SSW1 should be used as a high-yield and high-efficiency cultivation mode for winter wheat, and it was characterized with moderate root growth and distribution at the surface and upper soil layers but relatively large root amounts at the middle and lower soil layers. With this cultivation model, the growth peak of deep roots appeared later, and the post-peak attenuation decline was postponed. With this root distribution characteristic, water storage consumption at the middle and lower soil layers was effectively increased, much root biomass at late growth stages was sustained, water requirement during grain filling period was achieved, and water use efficiency was increased, resulting in the promoted dry matter productions and grain yields. In conclusion, the root proportions at deep soil layers can play partial roles in the potential of wheat grain yields. Improving the root distribution and absorption capacity at deep soil layers during cultivation helps obtain high grain yields and high water use efficiency.

Materials and methods
Study site. A field experiment was conducted in lime concretion black soil for two successive years from 2015 to 2017 at the 14th sub-farm of Shanghui County, Henan Province, China (33° 32′ N, 114° 29′ E). This experimental site is in a warm temperate continental monsoon climate, and the soil type is characterized with high viscosity, dry shrinkage and wet expansion, and shallow groundwater, as described in one previously published literature\textsuperscript{4}. In our study site, there exists the lime concretion at 60–80 cm soil layers in our study site, and deep soil layers are relatively soft. The wilting coefficient and the available water content of the soil were 8.02% and 23.42%, respectively. Moreover, the annual average temperature in this area was 14.5 °C, the accumulated sunshine duration was 2072.3 h, the average frost-free season was 223 d, and the annual average precipitation was 784.1 mm (meteorological data for 1997–2017, published by the Shanghui Meteorological Bureau). The nutrient content of the plough layer soil was measured in 2015 before wheat sowing. The values observed in the 0–20 cm soil layer were as follows: organic matter, 21.32 g·kg\textsuperscript{−1}; total nitrogen, 1.36 g·kg\textsuperscript{−1}; Olsen phosphorus, 16.83 mg·kg\textsuperscript{−1}; available potassium, 214.61 mg·kg\textsuperscript{−1}; and pH, 7.23. Those in the 20–40 cm soil layer were as follows: organic matter, 16.06 g·kg\textsuperscript{−1}; total nitrogen, 1.23 g·kg\textsuperscript{−1}; Olsen phosphorus, 8.58 mg·kg\textsuperscript{−1}; and available potassium, 163.26 mg·kg\textsuperscript{−1}. The soil bulk density data before and after tillage are shown in Supplementary Table S2 online.

The precipitation amounts during the wheat growing periods in 2015–2016 and 2016–2017 were 281.3 mm and 360.9 mm, respectively. Precipitation and irrigation amounts at different growth stages in the two years are shown in Supplementary Table S3 online. The field water-holding capacity is shown in Supplementary Table S4 online. Solar radiation and daily average air temperature during the wheat growing stages were 12.80 MJ·m\textsuperscript{−2}·d\textsuperscript{−1} and 9.52 °C (2015–2016), and 13.51 MJ·m\textsuperscript{−2}·d\textsuperscript{−1} and 10.81 °C (2016–2017), respectively.

Experimental design
Field experiment. The crop previously grown in the experimental field was corn, and the straw was crushed and returned to the field. Wheat cultivar Zhoumai 27 used in this experiment is a commercial cultivar, which has been authorized by the China government in 2011 to be permitted to plant in China. In both years, wheat sowing was delayed by continuous rain before sowing. For this reason, the growth practices, promoting deep rooting and maintaining the quantity and activity of the deep root system should be key to achieving high yield.

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Root parameters. Root samples were collected from the 0–100 cm soil layer in each plot at the jointing, heading, anthesis, filling and maturity stages, and every 20 cm of depth was regarded as one soil layer. The sampling method was the same as that used by Wang et al.7. After processing, the root parameters such as root length, root surface area and root volume in different soil layers were obtained by root system analysis software (WinRHIZO 2008). The root was dried with absorbent paper and then placed in an oven at a constant temperature of 80 °C to obtain the root dry weight. The dry root weight density (DRWD, g·m⁻³), root length density (RLD, cm·cm⁻³), root surface area per unit area (RSA, m²·ha⁻¹) and root volume per unit area (RV, m³·ha⁻¹) in different soil layers were determined by Eqs. (1), (2), (3) and (4), respectively49-52:

\[
DRWD = \frac{M}{V} \times 10^6
\]  
(1)

\[
RLD = \frac{L}{V}
\]  
(2)

\[
RSA = \frac{A}{S} \times 10^8
\]  
(3)

\[
RV = \frac{V'}{S} \times 10^8
\]  
(4)

Total dry root weight (TDRW, g·m⁻²), total root length (TRL, cm·cm⁻²), total root surface area (TRSA, m²·ha⁻¹) and total root volume (TRV, m³·ha⁻¹) refer to the sums of root weight, root length, RSA, and RV per unit soil area in different soil layers53. They were determined by Eqs. (5), (6), (7) and (8), respectively:

\[
TDRW = \sum_{i=1}^{n} M_i/S \times 10^4
\]  
(5)

\[
TRL = \sum_{i=1}^{n} L_i/S
\]  
(6)

\[
TRSA = \sum_{i=1}^{n} A_i/S \times 10^8
\]  
(7)

\[
TRV = \sum_{i=1}^{n} V'_i/S \times 10^8
\]  
(8)

where, M, L, A, V, V' and S are root dry weight (g), root length (cm), root surface area (m²), root volume (cm³), soil volume (cm⁻³) and soil area (cm²), respectively. The soil layer number is represented by i. There are five layers in total, and n is the total number of soil layers. Moreover, \(M_i\), \(L_i\), \(A_i\) and \(V'_i\) are the root dry weight, root length, root surface area and root volume of the i-th layer, respectively.
Grain yields and components. In each plot, the 6 m² (2 m × 3 m)-wheat plants were manually harvested to measure grain yields. The fixed sampling points for one-metre double rows of each treatment were investigated at maturity to obtain the spike number. Fifty representative stems were selected for each repeated treatment in each plot during the harvest period, packed into mesh bags, marked and then taken back to the laboratory to measure the kernel number per spike. Grain weight was measured after threshing and drying and converted into actual yield according to a 13% water content.

Yield contribution rate. Wheat samples from the soil column experiment were harvested at maturity to measure wheat yield and three factors of yield. No root cutting (CK), cutting of wheat roots at 40 cm below the surface (T-40) and cutting of roots at 80 cm below the surface (T-80) are represented by Y₀, Y₄₀ and Y₈₀, respectively. C₄₀, C₄₀–₈₀ and C₈₀ are the yield contribution rates of roots in the 0–40 cm, 40–80 cm, and below 80 cm soil layers, respectively. They were determined by Eqs. (9), (10) and (11), respectively:

\[
C_{40} = \frac{Y_{40}}{Y_0} \times 100
\]

\[
C_{40–80} = \left(\frac{Y_{80} - Y_{40}}{Y_0}\right) \times 100
\]

\[
C_{80} = \left(\frac{Y_0 - Y_{80}}{Y_0}\right) \times 100
\]

Statistical analysis. Excel 2013 and Origin 2018 were used for data processing and graphing. Statistical analyses, significance tests (Duncan’s test) and correlation analysis (Pearson) were performed using SPSS 23.0.
The authors declare no competing interests.

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Author contributions

Y.W. and T.G. designed the research; J.W. wrote the main manuscript text; J.W. and Z.W. analyzed the data; Z.W. drew the figures; F.G. and H.L. collected the data; G.K. and W.F. contributed to the revised manuscript. All authors discussed the results and commented on the manuscript at all stages.

Competing interests

The authors declare no competing interests.
