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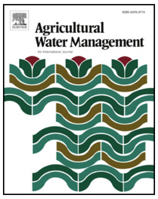

Using stable isotopes to quantify water uptake from different soil layers and water use efficiency of wheat under long-term tillage and straw return practices

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ABSTRACT

To quantify the water absorbed by wheat in different soil layers and improve both wheat yields and water use efficiency (WUE), a 2-year conservation tillage and straw treatment experiment was implemented. This experiment involved four tillage methods, conventional tillage (C), subsoiling (S), rotary tillage (R), and no-tillage (N), and two straw treatments, straw return (W) and no straw return (0). The hydrogen, oxygen and carbon stable isotope method was used to evaluate the water source, grain yield, dry matter quantity and WUE of wheat as well as the relationships between $\Delta^{13}C$ values of the wheat leaves, stems, ears and yield and the WUE under different tillage and straw treatment methods at the jointing and harvest stages. The results indicated that wheat water uptake occurred mainly within the 0–20 cm (86.22 %) soil layer at the jointing stage and within the 0–20 (56.36 %) and 20–40 cm (38.74 %) soil layers at the harvest stage. Compared with those in the C-0 treatment, the dry matter quantity in the S–W treatment increased by 14.86 % and 14.20 % respectively, at the jointing stage and the harvest stage; the grain yield in the S-W treatment significantly increased by 18 % at the harvest stage ($P < 0.05$); the WUE_t and WUE_i in the S-W treatment significantly increased by 46.21 % and 45.31 %, respectively, at the jointing stage ($P < 0.05$); and the WUE_y increased by 5.69 % and 5.54 %, respectively, at the jointing stage and the harvest stage. The $\Delta^{13}C$ vales of the wheat leaves, stems and ears were positively correlated with the yield, dry matter quantity and WUE of wheat. In conclusion, subsoiling with straw return should be adopted as a promising strategy for improving both wheat productivity and WUE and for retaining soil water availability. The $\Delta^{13}C$ value of wheat organs can be used to indicate changes in wheat yield and WUE.

1. Introduction

In recent decades, increasing amounts of attention have been paid to the interactions between precipitation, soil water, groundwater and plant stem water (Wang et al., 2009). However, estimating the important effects of these fluxes on the improvement of crop water use efficiency (WUE) by quantifying the water fluxes of evaporation, transpiration and drainage at the soil-plant-atmosphere interface is difficult. The water absorption of the root system plays a very important role in the water interaction in the soil-plant-atmosphere ecosystem (Asbjornsen et al., 2007). Absorbing water by their root system and transpiration of leaves, plants regulate the rate and amount of water reentering the land water cycle, thus regulating the energy flow and cycle of materials in an ecosystem. In contrast, plant metabolism and soil resources affect plant physiological functions via soil moisture availability.

Hydrogen and oxygen stable isotope methods have received extensive amounts of attention for predicting water sources in forest, grassland and farmland ecosystems (Yang et al., 2015). Because water does not exchange with the external environment while being transported from the soil to the plant root system and through xylem vessels, usually no isotope fractionation occurs (White et al., 1985). The isotopic composition of water in the xylem of plant stems can reflect the isotope information of the source of that water (Zimmermann et al.,

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1967). Therefore, the contribution of these sources to plants can be determined by comparing the stable isotopes of hydrogen and oxygen between plant stem water and possible water sources (Brunel et al., 1995). The difference between isotopes from the different water source is caused by isotope fractionation, while the main causes of isotope fractionation are physicochemical processes such as evaporation, falling and infiltration (Asbjornsen et al., 2007). Soil water is the most important source of water for plants. Because the nature of the soil itself, such as particle size and porosity, there are differences in stable isotopes of hydrogen and oxygen at different soil layers. Generally speaking, the moisture in the surface soil is relatively high in evaporation intensity due to easier contact with air, thus resulting in stable isotope fractionation of hydrogen and oxygen. According to the above theory, "light" molecules are preferentially evaporated, so the surface soil water is more enriched with heavier stable isotopes of hydrogen and oxygen than the deeper soil water. Therefore, the stable isotope composition of hydrogen and oxygen in soil water at different soil depths is significantly different, and is relatively stable until the deeper soil layer (Asbjornsen et al., 2007). It is precisely because of various physical and chemical processes in nature that hydrogen and oxygen stable isotopes from different sources are fractionated to different degrees, which provides a research basis for quantitatively distinguishing different water sources of plants (Burgess et al., 2000). Most relevant studies have used the direct comparison method (Sekiya and Yano, 2002) and the equal source mixed model to determine plant water sources (Phillips et al., 2005). A two-layer mixed model was used to study the water supply status of pine trees during different seasons in central and southern Texas. The results showed that the main use of groundwater occurred during the dry and hot seasons, whereas soil moisture was mainly used during the cold and wet seasons (McCole and Stern, 2007). Asbjornsen et al. (2007) reported that maize and prairie plants obtain 45 % and 36 % of their water from the 0–20 cm soil layer, respectively. The contribution of soil moisture within a depth of 0–100 cm was studied by the use of a stable isotope technique. Moreover, soil water at different growth stages was absorbed mainly within the 0–40 cm soil layer (Zhang et al., 2011)

WUE is an important index that reflects the ability of plants to adapt to drought environments. The WUE can be divided into three forms: WUE_t (photosynthetic rate / transpiration rate, A / Tr), WUE at the leaf level (WUE_i; photosynthetic rate / stomatal conductance, A / g_s) (Erice et al., 2007), and WUE at the yield level (WUE_y; yield / crop water consumption, Y / ET). Jones (2004) reported that $\Delta^{13}C$ can be used to predict the WUE of C3 plants. The $\Delta^{13}C$ values of different plant organs can indicate the WUE at different levels. For example, Condon et al. (1987) pointed out that, in South Australia, grain $\Delta^{13}C$ (ΔE) was positively correlated with wheat grain yield. In addition, Farquhar et al. (1989a) reported a negative correlation between $\Delta^{13}C$ (ΔL) and WUE_i in C3 plants.

With respect to improving crop yield and WUE, land use methods are very important. In agriculture, conservation tillage is a new type of tillage different from traditional tillage in terms of operation, time and production cost, mainly including reduced tillage, no-tillage and straw mulching, etc (SCSA, 1982). Conservation tillage can help preserve soil and water, increase soil moisture contents, and improve soil quality and crop yields, all of which are conducive to the sustainable development of agricultural production (Balwinder-Singh et al., 2011). Conventional tillage reduces soil water retention and disrupts soil structural stability, biological activity, and nutrient supplies and storage (Bissett et al., 2013). Especially in wheat fields, conventional tillage leads to a relatively narrow plow layer and hinders the flow of air and water, thus inhibiting root growth and reducing yields (Huang et al., 2012). As such, the sustainable development of agriculture in the Huang-Huai-Hai region of China has been adversely affected by the destruction of soil structure (Latifmanesh et al., 2018), thus reducing the WUE. Compared with conventional tillage, subsoiling can reduce the effects of soil compaction on crop yield (Jennings et al., 2012) while increasing root elongation and the effects of moisture on crop growth (Lampurlanés et al., 2001). In addition, no-tillage and subsoiling with straw mulch usually result in an increase in chlorophyll pigment content and net photosynthetic rate in flag leaves (Li et al., 2006b). Therefore, conservation tillage can improve soil water storage capacity and crop yield, thus increasing economic benefits (Gicheru et al., 2004). However, (Guzha, 2004) reported that the yield of wheat under reduced tillage and no-tillage conditions was lower than that under conventional tillage conditions.

Many studies have investigated WUE in response to irrigation and water-deficit treatments, but relatively little research exists on the WUE of crops under different tillage methods. Therefore, four tillage and two straw treatment methods were used as part of a 16-year long-term experiment. The hydrogen and oxygen isotope values of the precipitation, groundwater, irrigation water, soil water and stem water as well as the carbon isotope values of the leaves, stems and ears of the wheat plants were determined at the jointing and harvest stages. Moreover, the soil water content, dry matter quantity of aboveground plant parts and wheat yields were determined. The objectives of this study were to (1) quantify the contributions of water from different soil layers to wheat; (2) compare the crop yields and WUE values under different tillage and straw treatments; and (3) evaluate the relationships between the $\Delta^{13}C$ values of different wheat organs and crop yield, dry matter quantity and WUE.

2. Materials and methods

2.1. Trial site

This experiment was conducted at Tai'an (northern China, 36°09′N, 117°09′E, altitude 130 m above sea level), which has a typical temperate continental monsoon climate; the annual average temperature is 12.82 °C, and the annual average rainfall was 956.69 mm from October 2017 to June 2019. The soil is classified as Cambisols (FAO-UNESCO, 1988). The basic physical and chemical properties of the soil within the 0–20 cm layer are shown in Table 1. The mean precipitation and atmospheric temperature every month in wheat season during 2017–2019 were showed in Table 2. The sample collection time was shown in Table 3. The soil water content before sowing in 2017 and 2018 was shown in Table 4.

2.2. Experimental design

The experimental site was cropped with a typical rotation of winter wheat (Jimai-22) and summer maize (Zhengdan-958) in northern China. This study was based on a 16-year-long conservation tillage experiment that began in 2002 and was established as a split-plot design replicated three times. Each plot was 15 m long and 4 m wide. This experiment involved four tillage and two straw incorporation methods: conventional tillage (C-0), subsoiling (S-0), rotary tillage (R-0), and no-

Table 1
Basic characteristics of main soil physicochemical in 0 - 20 cm soil layer (2002).

Physical properties		Chemical properties	
Sand (%)	40	SOC (g kg^{-1})	7.19
Silt (%)	44	TN (g kg^{-1})	1.3
Clay (%)	16	TP (g kg^{-1})	8.09
BD (g cm^{-3})	1.43	TK (g kg^{-1})	2.16
pH	7.09	AN (mg kg^{-1})	108.8
Porosity (%)	51.59	AP (mg kg^{-1})	0.79
		AK (mg kg^{-1})	41.32

BD: Bulk density; SOC: Soil organic carbon; TN: Total nitrogen; TP: Total phosphorus; TK: Total potassium; AN: Available nitrogen; AP: Available phosphorus; AK: Available potassium.

Table 2
The mean precipitation and atmospheric temperature every month in wheat season during Oct/2017 - Jun/2019.

Time	Oct/2017	Nov/2017	Dec/2017	Jan/2018	Feb/2018	Mar/2018	Apr/2018	May/2018	Jun/2018	Oct/2018	Nov/2018	Dec/2018	Jan/2019	Feb/2019	Mar/2019	Apr/2019	May/2019	Jun/2019
Precipitation (mm)	48.7	9.9	28.8	11.1	21.6	213.9	168.46	536.2	489.3	105.9	75.7	16.2	5.29	9.47	16.3	31.28	48.42	86.01
Temperature (°C)	14.5	7	1.5	- 1.5	1.5	10.5	16.5	21.5	27	14	8.5	0	0.5	2	10	15	21.5	22.5

tillage (N-0) without straw return as well as conventional tillage (C-W), subsoiling (S-W), rotary tillage (R-W), no-tillage (N-W) with straw return.

All kinds of tillage practices were carried out before wheat sowing, and no tillage was performed during the maize season. The specific operating procedures were as follows:

(1) conventional tillage (C): maize harvesting-whole straw returning/no straw returning–stubbling–applying basal fertilizer–plowing with a moldboard plow (25 − 35 cm)–rotary with a rotary cultivator–ridging–sowing wheat;
(2) subsoiling (S): maize harvesting–whole straw returning/no straw returning–stubbling–applying basal fertilizer–subsoiling with a deep shovel (40 − 45 cm)–rotary with a rotary cultivator–ridging–sowing wheat;
(3) rotary tillage (R): maize harvesting–whole straw returning/no straw returning–stubbling–applying basal fertilizer–rotary with a rotary cultivator (10 − 15 cm)–ridging– sowing wheat;
(4) no-tillage (N): maize harvesting–whole straw returning/no straw returning-stubbling–applying basal fertilizer–ridging–sowing wheat.

The aim of rotary after tillage in above procedures before sowing is to make the ground flat, and make the sowing depth consistent and ensure the emergence of seedlings to be neat and uniform. Ridges was used to eliminate the interaction between plots between. Winter wheat was generally sown between 10 and 15 October and harvested between 6 and 10 June the following year. A basal fertilizer that consisted of 225 kg N ha^{-1}, 180 kg P ha^{-1}, and 180 kg K ha^{-1} was applied before sowing, and 110 kg N ha^{-1} was applied at the jointing stage together with 75 mm of irrigation.

2.3. *Sample collection and determination*

2.3.1. *Determination of soil water content*

The soil sampling time was shown in Table 3. Soil samples of 0–20, 20–40, 40–60, 60–80, and 80–100 cm were collected with three replications. After the fresh soil was weighed (FS), the soil was dried in the oven until constant weight, and then the dry soil was weighed (DS). The soil water content was calculated as follows (Toumi et al., 2016):

$$\text{Soil water content (\%)} = \text{FS} / \text{DS} \times 100 \qquad (1)$$

2.3.2. *Determination of delta (δ)D and δ^{18}O contents in the soil water*

A minimum of three evaporating dishes (each containing 2 mm of liquid paraffin at the bottom to prevent water evaporation) were put in the experimental field to collect precipitation. Irrigation water was collected every 15 min and 6 times per irrigation event. Soil and wheat base samples were collected in the early morning at the jointing and harvest stages, both in the presence and absence of precipitation and irrigation for at least three days. Soil samples within the 0–20, 20–40, 40–60, 60–80, and 80–100 cm layers and at 200 cm (considered groundwater in this experiment), as well as wheat stem base samples, were collected. All the samples were immediately placed in capped glass bottles that were subsequently sealed with Parafilm. The soil and wheat samples were preserved at − 20 °C, and precipitation and irrigation water samples were stored at 4 °C. The water in the soil and plant samples was fully extracted by a liquid water low-temperature vacuum extraction system (LI-2100, LICA United Technology Limited, Beijing, China). After the extraction was completed, the water hydrogen and oxygen isotopes were determined by an Isotope Ratio Mass Spectrometer (Thermo Electron, model Delta V Advantage, Bremen, Germany).

Table 3
The dates and the year for the samplings.

Sample	Sampling time									
Precipitation	Mar/28/2018	Apr/21/2018	Apr/30/2018	May/15/2018	May/21/2018	Mar/20/2019	Apr/24/2019	Apr/27/2019	May/12/2019	May/26/2019
Irrigation	Oct/17/2017	Mar/25/2018	Oct/16/2018	Mar/28/2019						
Soil and wheat	Apr/9/2018	Jun/3/2018	Apr/11/2019	Jun/2/2019						

Table 4
The soil water content before sowing wheat in Oct/2017 - Jun/2019 (%).

Time	Treatment	Soil layer (cm)				
		0–20	20–40	40–60	60–80	80–100
Oct-2017	C-0	10.46	12.32	15.05	15.61	15.38
	C-W	10.98	12.39	15.94	16.03	15.80
	S-0	11.92	12.81	15.72	15.89	15.63
	S–W0	12.64	12.93	16.03	16.25	15.73
	R-0	13.26	13.91	16.22	16.49	15.94
	R-W	13.57	14.37	16.34	16.60	16.01
	N-0	13.38	13.77	16.39	16.78	15.75
	N-W	13.88	14.50	16.57	16.91	15.90
Oct-2018	C-0	12.05	15.76	17.50	17.71	17.39
	C-W	13.53	15.68	18.18	18.32	17.42
	S-0	12.95	16.74	18.16	17.39	17.12
	S-W	14.32	16.92	18.00	17.65	16.97
	R-0	15.73	16.00	18.21	18.27	16.13
	R-W	16.29	16.80	17.95	18.59	16.96
	N-0	15.64	16.21	18.24	18.33	16.25
	N-W	16.31	16.94	18.34	18.67	16.49

C-0: conventional tillage without straw returning; C-W: conventional tillage with straw returning; S-0: subsoiling without straw returning; S-W: subsoiling with straw returning; R-0: rotary tillage without straw returning; R-W: rotary tillage with straw returning; N-0: no-tillage without straw returning; N-W: no-tillage with straw returning.

2.3.3. Determination of the gas exchange parameters, dry matter quantity, grain yield and $\delta^{13}C$ content of each organ in wheat

The photosynthetic rate (A), stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i) and transpiration rate (Tr) of wheat were measured by a LI-6400 photosynthetic instrument during the jointing stage. At the jointing and harvest stages, one square meter of wheat was selected in each treatment (with three replications); the plant material was collected from the selected area and returned to the laboratory, where it was first placed in an oven at 105 °C to destroy the living components and then dried to a constant weight at 75 °C to measure the amount of dry matter. Grain yield was measured at the harvest stage of wheat. After they were dried, the leaves, stems and ears of the wheat plants were separated, and their $\delta^{13}C$ values were measured by a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer.

2.4. Calculations and analytical methods

2.4.1. Contributions of water from different sources to wheat

The method was to use IsoSource software to calculate the contribution of different water sources to wheat. The fractional increment was set at 1 %, and the uncertainty level was set at 0.2. The sensitivity was analyzed with different fractional increments (0.5, 2 %) and at different uncertainty levels (0.1, 0.3, and 0.4). The results showed no significant differences in changes in fractional increment and uncertainty level.

2.4.2. Isotope ratio calculations

Natural ^{13}C abundance, expressed in δ units, which indicates the isotopic ratio of a sample relative to that of the Pee Dee Belemnite (PDB) standard, can be calculated as follows (Farquhar et al., 1989a):

$$\delta^{13}C(‰) = (R_{sample} / R_{standard} - 1) \times 1000 \tag{2}$$

where R_{sample} is the isotopic ratio of the study material and $R_{standard}$ is that of the reference standard (PDB).

Isotopic effects can also be expressed by isotopic discrimination values (Δ). Because CO_2 is the source of plant photosynthesis, its photosynthetic discrimination value can be described as follows (Martínez-Sancho et al., 2017):

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p / 1000) \tag{3}$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ of atmospheric CO_2 (–8‰) and $\delta^{13}C_p$ is the $\delta^{13}C$ of wheat leaves, stems or ears. The isotopic discrimination values of leaves, stems, and ears are represented by ΔL, ΔS and ΔE, respectively.

2.4.3. Determination of WUE

WUE_t refers to the amount of photosynthate assimilated per unit of water via leaf transpiration and depends on the ratio of leaf net A to the T_r. WUE_t represents the behavior of only a portion of the leaves of a plant based on instantaneous photosynthesis and transpiration. The formula is as follows (Farquhar et al., 1989a):

$$WUE_t = A / Tr \tag{4}$$

WUE_i refers to the ratio of A to g_s within plant leaves and reflects the inherent regulation of carbon absorption and water dissipation in leaves. This metric can be expressed as follows (Farquhar et al., 1989b):

$$WUE_i = A / g_s \tag{5}$$

According to Jones (2004), the WUEy of crops has been defined as various combinations of the ratio of harvested yield, aboveground biomass or total biomass to plant transpiration, evapotranspiration (ET) or total available water. In the present study, WUE_y was calculated as the percent grain yield divided by the amount of ET during the growing season (Hussain and Al-Jaloud, 1995) using the following equation (Huang et al., 2005):

$$ET = P + I + C + (SW_1 – SW_2) – D – R \tag{6}$$

$$WUE_y = Yield/ET \tag{7}$$

where ET (mm) is the evapotranspiration, P (mm) is the effective precipitation during the growing season, I (mm) is the irrigation, C (mm) is the upward flow of water into the root zone, SW_1 (mm) is the soil water content at the time at which the crop was sown, SW_2 (mm) is the soil water content at the time at which the crop was harvested, D is the downward drainage from the root zone, and R is the surface runoff. In the study area, the ground was flat, and the visual surface runoff could be considered null. The groundwater depth was below 4 m; the amount of groundwater recharge could therefore be considered negligible. The depth of infiltration was not greater than 2 m; thus, the depth of the leakage could be considered null. Therefore, C, D and R terms in the above equation could be ignored.

2.5. Statistical analyses

The figure data were analyzed and visualized by SigmaPlot (Ver.

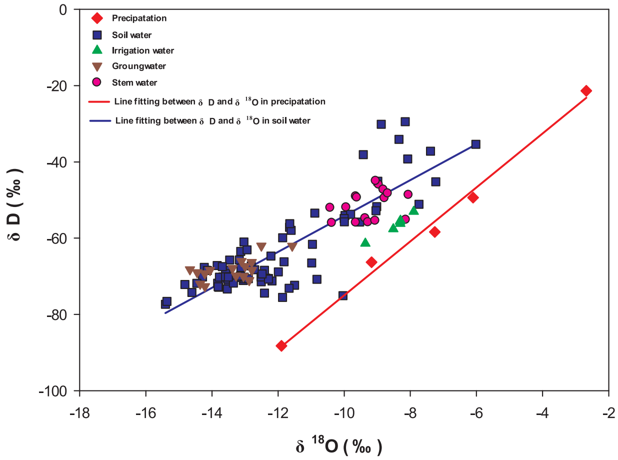

Fig. 1. δD–δ^{18}O relationship of different water sources during Oct/2017-Jun/2019.

12.5, Systat Software, Inc., San Jose, USA). Significant differences between treatments were assessed using SPSS 18.0 Statistical Analysis System software (SPSS, 2009) (Duncan's test at $P < 0.05$ and $P < 0.01$).

3. Results

3.1. Isotopic composition of water

The hydrogen isotope value (δD) of the precipitation water ranged from −88.24 to −21.36‰, with an average value of −56.73‰. The oxygen isotope value (δ^{18}O) ranged from −11.90 to −2.68‰, with an average value of −7.42‰ (Fig. 1). The variation range of hydrogen isotope value was higher than that of oxygen isotope. There was a significant linear relationship between hydrogen and oxygen in all water samples, and δD and δ^{18}O are evenly distributed in all water samples. The stable isotopic distribution of stem water of wheat was near the fitting line of the soil water, which indicated that the wheat stem water originated from soil water during this stage. The irrigation water originated from a well next to the experimental field. In the present study, water at a depth of 2 m was considered groundwater. The distribution of δD and δ^{18}O in the irrigation water and groundwater was relatively concentrated, while that in the precipitation water and soil water was more dispersed.

At the wheat jointing stage, the δD and δ^{18}O of the soil water ranged from −55.80 (R-0) to −45.19‰ (C-W) and −10 (N-W) to −7.23‰ (C-W), respectively, within the 0–20 cm soil layer. The δD and δ^{18}O of the soil ranged from −70.63 (S-W and N-W) to −65.06‰ (R-0) and −13.52 (C-W) to −12.34‰ (C-0), respectively, within the 20–100 cm soil layer (Fig. 2A). At the wheat harvest stage, the δD and δ^{18}O of the soil water ranged from −45.06 (C-W) to −29.45‰ (N-W) and −9.42 (R-0) to −6.02‰ (C-0), respectively, within the 0–20 cm soil layer. The δD and δ^{18}O of the soil ranged from −73.34 (S-W and N-W) to −65.07‰ (R-0) and −13.71 (C-W) to −11.51‰ (C-0), respectively, within the 20–100 cm soil layer (Fig. 2B). In the 20 − 100 cm soil layer, the treatments of the highest and lowest hydrogen and oxygen isotope values in the two periods were consistent. The line fitting between the δD and δ^{18}O of the soil water was calculated as $\delta D = 4.79\delta^{18}O{-}7.26$ ($R^2 = 0.702$, $P < 0.0001$). The δD of the soil water did not significantly differ between treatments (Table 5), while the δ^{18}O of the soil water in C-0 was significantly greater than that in other treatments ($P < 0.05$). The δD and δ^{18}O of the soil water within the 0–20 cm soil layer were significantly greater than those within the 20–100 cm soil layer ($P < 0.05$). With the soil depth, the hydrogen and oxygen isotope values decreased. This was because the seasonal variation of soil water input, the evaporation of surface layer or the difference between soil water and groundwater. Soil water produced obvious isotopic composition gradient with the change of soil depth.

3.2. Wheat water uptake from different soil layer

At the jointing stage, wheat absorbed water mainly within the 0–20 cm soil layer; the contribution of this soil profile was 86.22 % and was significantly greater than that of the other soil profiles ($P < 0.05$). At the harvest stage, wheat absorbed water mainly within the 0–20 (56.36 %) and 20–40 cm (38.74 %) soil layers (Table 6). From jointing stage to harvest stage, the depth of soil water used by wheat gradually deepened.

3.3. Dry matter quantity, grain yield and WUE of wheat at the jointing and harvest stages

Compared with that in the C-0 treatment, the wheat dry matter quantity in the S-W treatment increased by 14.20 % and 5.54 % at the jointing and harvest stages, respectively (Table 7). Moreover, the dry matter quantity in the N-0 treatment was significantly lower than that in the C-0 treatment at both stages ($P < 0.05$). Similarly, compared with that in the C-0 treatment, the grain yield in the S-W treatment increased by 18 %, and the grain yield in the N-0 treatment was the lowest at the harvest stage. The instantaneous water use efficiency (WUE$_t$) in the S-W treatment was significantly greater than that in the other treatments ($P < 0.05$) (except C-W), and compared with that in the C-0 treatment, it increased by 46.21 %; in addition, the WUE$_t$ in the N-0 treatment was the lowest at the jointing stage. The intrinsic water use efficiency (WUE$_i$) in the S-W treatment was significantly greater than that in the other treatments ($P < 0.05$), and compared with that in the C-0 treatment, it increased by 45.31 %; in addition, the WUE$_i$ in the N-0 treatment was significantly lower than that in the C-0 treatment ($P < 0.05$) at the jointing stage. Compared with that in the C-0 treatment, the yield water use efficiency (WUE$_y$) in the S-W treatment increased by 14.86 % and 5.69 % at the jointing and harvest stages, respectively, and the WUE$_y$ in the N-0 treatment was significantly lower than that in the other treatments at both stages ($P < 0.05$). Therefore, compared with other treatments, S-W treatment can improve dry matter quality, grain yield and water use efficiency. However, after long-term no-tillage treatment, these indexes were significantly reduced.

3.4. Relationships between ΔL, ΔS, and ΔE and grain yield, dry matter quantity and WUE under different tillage and straw treatments

As shown in Fig. 3, there were significant positive correlations between the carbon isotopic discrimination of leaves (ΔL) and grain yield ($P < 0.05$), dry matter quantity ($P < 0.01$), and WUE$_y$ ($P < 0.01$). The carbon isotopic discrimination of stems (ΔS) was significantly positively correlated with grain yield, dry matter quantity, WUE$_i$ and WUE$_y$ at the $P < 0.01$ level and with WUE$_t$ at the $P < 0.05$ level. In addition, there were significant positive correlations between the carbon isotopic discrimination of ears (ΔE) and WUE$_t$ and WUE$_i$ at the $P < 0.05$ level and with dry matter quantity and WUE$_y$ at the $P < 0.01$ level. Therefore, it was possible that the carbon isotope in different plant organs can indicate changes of dry matter, grain yield and WUE.

4. Discussion

4.1. Contributions of water from different soil layers to wheat

In the present study, the δD and δ^{18}O of the topsoil water were significantly different from those of the deep layer. Moreover, the δ^{18}O of soil water in 0–20 cm soil layer were significantly different among treatments. This difference may be the result of situation during precipitation. Isotope fractionation occurs due to the influence of

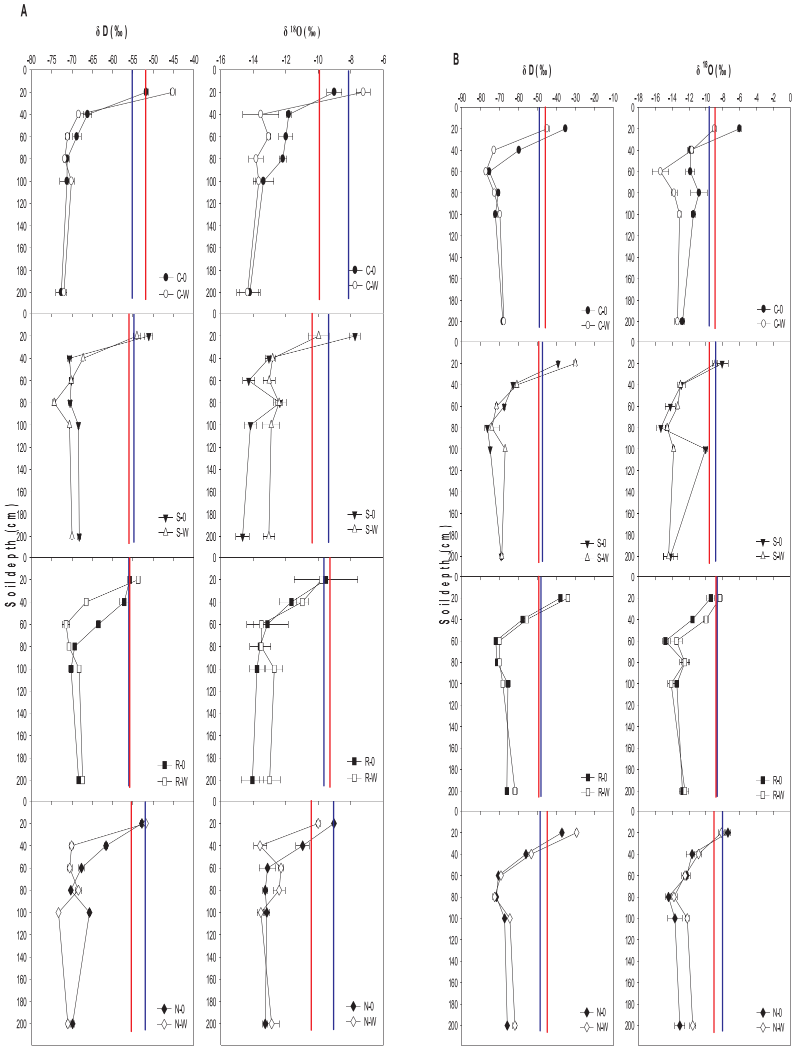

Fig. 2. The mean δ D and δ ^{18}O in soil water and stem water under different tillage methods and straw treatments during jointing (A) and harvest (B) stages during Oct/2017-Jun/2019. Red line represents δ D or δ ^{18}O of stem water under without straw returning (−0), blue line represents δ D or δ ^{18}O of stem water under with straw returning (-W). C-0: conventional tillage without straw returning; C-W: conventional tillage with straw returning; S-0: subsoiling without straw returning; S-W: subsoiling with straw returning; R-0: rotary tillage without straw returning; R-W: rotary tillage with straw returning; N-0: no-tillage without straw returning; N-W: no-tillage with straw returning (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5
Effects of different treatments and soil depth on mean δD and δ^{18}O at the jointing and harvest stages of wheat during Oct/2017 - Jun/2019.

Treatment	δD (‰)	δ^{18}O (‰)
C-0	- 64.60 a	- 11.09 a
C-W	- 66.78 a	- 12.48 b
S-0	- 65.53 a	- 12.28 b
S-W	- 64.36 a	- 12.54 b
R-0	- 62.40 a	- 12.40 b
R-W	- 63.21 a	- 11.95 b
N-0	- 62.42 a	- 11.95 b
N-W	- 62.96 a	- 11.99 b
Soil depth (cm)		
0-20	- 44.34 a	- 8.62 a
20-40	- 63.17 b	- 12.03 b
40-60	- 70.70 c	- 13.30 c
60-80	- 71.89 c	- 13.25 c
80-100	- 69.48 c	- 13.11 c

C-0: conventional tillage without straw returning; C-W: conventional tillage with straw returning; S-0: subsoiling without straw returning; S-W: subsoiling with straw returning; R-0: rotary tillage without straw returning; R-W: rotary tillage with straw returning; N-0: no-tillage without straw returning; N-W: no-tillage with straw returning. Different letters in each column indicate significant differences between different treatments and soil layers ($P < 0.05$; Duncan's test).

Table 6
The mean contribution of water from different soil strata on winter wheat at jointing and harvest stages during Oct/2017 - Jun/2019.

Soil layer (cm)	Contribution rate (%)	
	Jointing stage	Harvest stage
0–20	86.22 a	56.36 a
20–40	5.87 b	38.74 b
40–60	2.71 cd	1.92 c
60–80	4.27 bc	0.33 c
80–100	0.93 d	2.65 c

Different letters indicate significant differences between different water sources ($P < 0.05$; Dancan's test).

temperature, elevation and other factors, resulting in differences in isotopes in precipitation in different time and space (Maguas and Griffiths, 2003). The spatio-temporal difference of hydrogen and oxygen isotopes in precipitation will lead to the spatio-temporal difference of soil water, surface water, groundwater and plant water. In addition, this difference may be because changes in δD and δ^{18}O caused by soil evaporation (English et al., 2007; Wang et al., 2010; Ma and Song, 2016). In the process of soil evaporation, light isotope molecules evaporate upward first, so the concentration of heavier oxygen isotope in the surface soil becomes larger. Soil evaporation increased the concentration of ^{18}O in shallow soil throughout a long period of time before irrigation (Allison and Leaney, 1982). Because of the limited

Table 7
Analysis of mean dry matter quantity, grain yield and water use efficiency under different tillage and straw methods at jointing and harvest stages of wheat during Oct/2017 - Jun/2019.

Growth stage	Treatment	Dry matter ($t\ ha^{-1}$)	Grain yield ($t\ ha^{-1}$)	WUE_t ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	WUE_i ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	WUE_y ($kg\ ha^{-1}\ mm^{-1}$)
Jointing stage	C-0	3.52 ab	–	4.35 b	73.89 bc	9.62 bc
	C-W	3.73 ab	–	5.12 ab	89.50 b	10.21 ab
	S-0	3.29 abc	–	5.02 b	81.28 bc	8.96 cd
	S-W	4.02 a	–	6.36 a	107.37 a	11.05 a
	R-0	3.32 abc	–	4.30 b	65.24 cd	9.07 cd
	R-W	3.74 ab	–	4.56 b	73.96 bc	10.18 ab
	N-0	2.60 c	–	4.02 b	54.23 d	7.04 e
	N-W	3.10 bc	–	4.03 b	69.03 cd	8.44 d
Harvest stage	C-0	19.30 abc	5.50 b	–	–	12.31 abc
	C-W	19.82 ab	6.42 a	–	–	12.66 ab
	S-0	19.92 ab	6.47 a	–	–	12.70 ab
	S-W	20.37 a	6.49 a	–	–	13.01 a
	R-0	16.68 d	5.51 b	–	–	10.70 d
	R-W	18.70 bc	5.57 b	–	–	11.93 bc
	N-0	14.60 e	4.73 c	–	–	9.33 e
	N-W	18.33 c	5.19 c	–	–	11.67 c

C-0: conventional tillage without straw returning; C-W: conventional tillage with straw returning; S-0: subsoiling without straw returning; S–W: subsoiling with straw returning; R-0: rotary tillage without straw returning; R-W: rotary tillage with straw returning; N-0: no-tillage without straw returning; N-W: no-tillage with straw returning. WUE_t (photosynthesis rate/transpiration rate, A/T_r), WUE_i (photosynthesis rate/stomatal conductance of CO_2, A/g_s), WUE_y (yield/crop water consumption, Y/ET_c), ET_c (water consumption). Different letters in each column indicate significant differences between different treatments ($P < 0.05$; Duncan's test).

precipitation, irrigation water is very important to the growth of winter wheat under low-moisture conditions (Guan et al., 2015). Wu et al. (2016) obtained the same results when studying the water absorption of maize.

Previous studies have shown that winter wheat absorbs mainly shallow water (0–20 cm) during most growth stages, whereas at the anthesis and filling stages, soil water is absorbed mainly within 20–40 cm (Zhao et al., 2018). In the present study, wheat roots mainly absorbed water of 0–20 and 0–40 cm soil layers at jointing stage and harvest stage, respectively. These phenomena may occur because most wheat roots grow within the 0–40 cm soil layer (Li et al., 2006a), and the dry root weight density of winter wheat is positively correlated with the contribution of water uptake (Zhao et al., 2018). However, in the late stage of wheat growth, there may be two reasons for the decrease of water absorption ratio in shallow layer, one was that the roots grew deeper with the growth of plants which made it possible for the plants to use more water from the deeper soil layers, another possible reason is the senescence of the root system on the surface reducing the water absorption from shallow layer (Yang et al., 2018).

4.2. *Effects of different tillage and straw treatments on the grain yield and WUE of wheat at the jointing and harvest stages*

Wang et al. (2004) reported that, compared with those under conventional tillage, the yield and WUE of wheat under subsoiling increased significantly by 18.8 % and 16.8 %, respectively. Zhang et al. (2013) reported that crop yields and WUE increased by 19.2 % and 10.1 %, respectively, under subsoiling compared with conventional tillage on the Loess Plateau. In addition, because the retention of crop residue on the soil surface, the evaporation of water was reduced, and the WUE increased (Jalota et al., 2000). In the present study, the δD and $\delta^{18}O$ values of no straw returning were higher than those of straw returning, which also indicated that the evaporation of soil water under straw returning would reduce. Su et al. (2007) reported that no-tillage and subsoiling were the best tillage methods for increasing water reserves, wheat yields, WUE and energy conservation. In this present study, however, the grain yield and WUE of the subsoiling with straw return were the highest, but the no-tillage without straw return was the lowest. Because long-term continuous no-tillage can easily lead to soil compaction (Raper et al., 1994), and soil compaction can limit the infiltration of rainwater and the absorption of deep soil moisture by crops, which will decrease crop yield (Unger and Kaspar, 1994). In this present study, higher δD and $\delta^{18}O$ values of deep soil water under no-tillage with no straw returning condition may resulted by lower rainwater infiltration and water deficit. In addition, rotary tillage reduces soil plow layer thickness and decreases the infiltration quantity of water, thus reducing wheat yields (Bengough et al., 2006). The δD and $\delta^{18}O$ values of deep soil water under rotary tillage were also higher than those of conventional tillage and subsoiling, which also indicated that there was water deficit in deep soil layers.

4.3. *Relationships between ΔL, ΔS, and ΔE and grain yield, dry matter quantity and WUE under different tillage and straw treatments*

Different crops or crop organs were found to have different isotopic compositions (Brugnoli and Farquhar, 2000). Merah et al. (2001) reported significant positive correlations between ΔE and wheat yields and between ΔL and wheat yields. In the present study, ΔE was positively correlated with grain yield, and the ΔL was positively correlated with WUE_t and WUE_i. However, there were significant negative correlations between ΔL and WUE_t and WUE_i in wheat under water-deficit conditions (Cabrera-Bosquet et al., 2007). Studies have shown that ΔE can be used to compare WUE_y and ET, because ΔE can reflect the effects of environmental factors such as water change on plant (Cui et al., 2009). In rice, a positive correlation between ΔE and WUE_y under different water-deficit conditions was reported (Andrea et al., 2006). Another study has shown that the ΔL and ΔE of rice are significantly negatively correlated with WUE_y (Anyia et al., 2007). Cui et al. (2009) showed that ΔL had better indicator for WUE_y and ΔE for yield. In this study, however, ΔE was the worst indicator to grain yield compared with ΔL or ΔS. These different results may be related to different experimental treatments, crop species, and climatic environments. In a word, the $\Delta^{13}C$ of different organs, includingΔL, ΔS, and ΔE, can compare the response of wheat to tillage and straw returning methods, and then compared the WUE, thus indirectly indicating crop yield and water use efficiency.

5. Conclusions

By the use of the hydrogen and oxygen stable isotope method, this study showed that, at the jointing stage, wheat absorbed water mainly from the 0–20 cm soil layer and that, at the harvest stage, soil water use

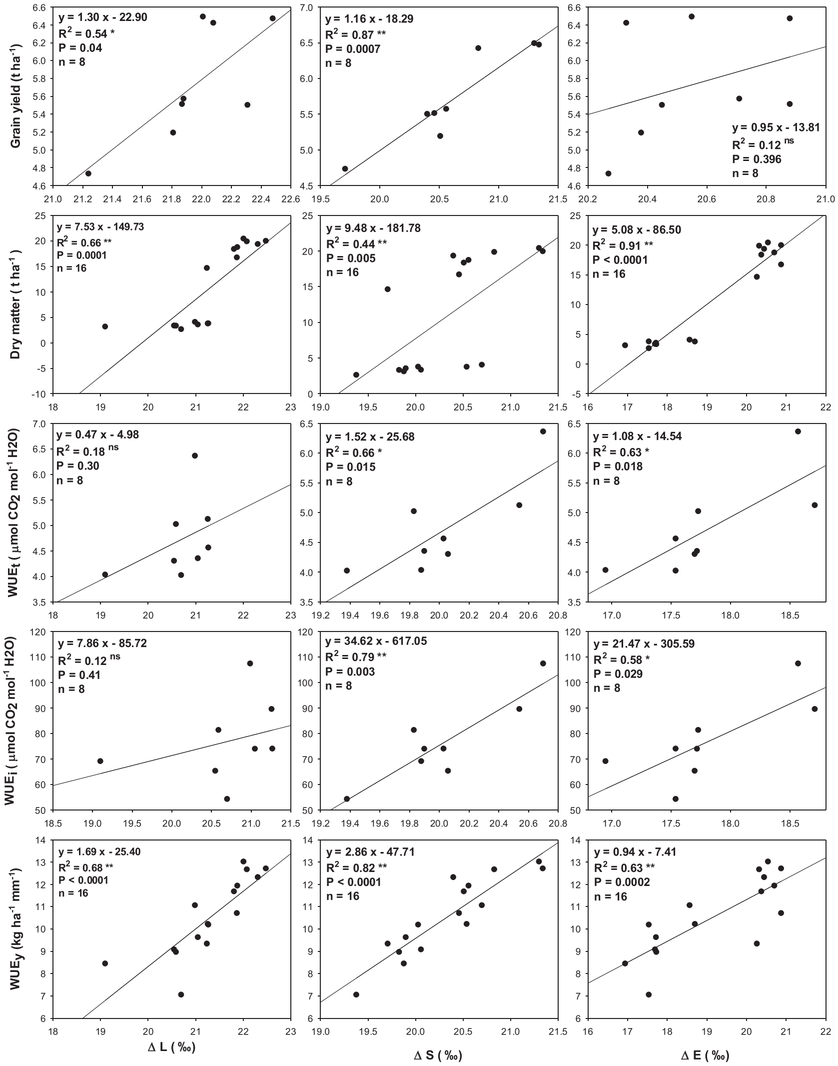

Fig. 3. The relationship between ΔL, ΔS, ΔE and grain yield, dry matter, WUE_t, WUE_i, WUE_y under different tillage methods and straw treatments during jointing and harvest stages during Oct/2017-Jun/2019. WUE_t: photosynthesis rate/transpiration rate, (A/T_r); WUE_i: photosynthesis rate/stomatal conductance of CO_2, (A/g_s); WUE_y: yield/crop water consumption, (Y/ET_c); Δ L: ^{13}C discrimination value of wheat leaf; Δ S: ^{13}C discrimination value of wheat stem; Δ E: ^{13}C discrimination value of wheat ear. * Significant difference at $P < 0.05$; ** Significant difference at $P < 0.01$; ^{ns} No significant difference.

within the 0–20 cm decreased while 20–40 cm layers increased.

The results of a 16-year-long tillage experiment showed that the dry matter quantity, grain yield and WUE of wheat significantly improved under subsoiling with straw return compared with other tillage treatments. Under conditions of no-tillage with or without straw return, the wheat yield, dry matter quantity and WUE were relatively low. Therefore, subsoiling is conducive to increasing wheat yields and improving WUE.

The $\Delta^{13}C$ values of the wheat leaves, stems and ears were significantly positively correlated with the yield, dry matter quantity and WUE of wheat at the $P < 0.05$ or 0.01 level. Positive correlations between ΔL and WUE_t and WUE_i and between ΔE and grain yield were recorded. Thus, the $\Delta^{13}C$ values of wheat organs can be used to indicate changes and differences in the yield and WUE.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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