Effective root depth and water uptake ability of winter wheat by using water stable isotopes in the Loess Plateau of China

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Abstract: A field experiment using PVC growth tubes was conducted in the Loess Plateau of China to determine the effective root depth (ERD) of winter wheat and its relationship with root distributions and soil water conditions. The water stable isotopes technique was used to estimate the water uptake contributions of different root depths during the growth stages. On the basis of IsoSource and the Romero-Saltos model, the ERD was 0-40 cm in the majority of the growth stage. However, in the heading and filling stages, the ERD could reach 60%-75% of the maximum root depth. Furthermore, the contributions to water uptake of different root depths were correlated with variations in soil water and root length density (r=0.395 and 0.368, respectively; p<0.05). However, by path analysis, the low decisive coefficient indicated that root distribution and soil water content did not always follow the same trend as water uptake. The conclusions of this study can help with understanding winter wheat water uptake mechanisms in arid and semi-arid regions and increasing water use efficiency.

Keywords: effective root depth, water stable isotopes, water uptake, root distribution, soil water content
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1 Introduction

Understanding the root structure and function in the root-soil-water system, in particular the interaction between root and water, may lead to the next Green Revolution[1]. Among the root parameters that are commonly measured, determining the effective root depth (ERD), which is defined as "the upper portion of root zone where plants get most of their water"[2], is important to (1) identify the soil volume in which plants can potentially extract water[3,4], (2) establish water uptake and transport patterns in the soil-plant-atmosphere continuum system[5-8], and (3) select appropriate irrigation depths and representative monitoring locations for soil water[9-12].

Studies indicate that the ERD is largely affected by root water uptake strategies, and it has traditionally been studied by excavating plant root distributions[2,13-15]. Evans et al.[2] proposed that the ERD should be estimated as one-half of the maximum root depth (MRD). Nevertheless, some studies concluded the root length density (RLD) was more helpful for estimating the ERD[16,17]. However, research has demonstrated that the root distribution may not always match the root water uptake pattern, for example, adult riparian trees preferred to rely on dependable groundwater resources[18,19], even though their roots were widely distributed in the surface

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soil. Similarly, most of the roots of wheat were concentrated in the upper soil layer, but the development of deep roots was favorable for soil water use during the grain filling[20-22]. Thus, it is better to determine the ERD not only from the root morphology, but also using physiological processes[23].

The technique based on water stable isotopes is an innovative approach for characterizing the ERD by analyzing the signatures of hydrogen and oxygen stable isotopes (δ^2H and δ^{18}O) of plant water and vertical gradients of soil water[19,23-25]. No isotopic fractionation of δ^2H and δ^{18}O was assumed to occur during absorption and transportation of water by roots[26], with the exception of some specific salt-tolerant plants[27]. Hence, the δ^2H and δ^{18}O values of plant xylem water can naturally represent the stable isotopic composition at the probable depth of root water uptake. Many studies have been conducted on the main ERD of forests and grasslands by directly comparing δ^2H and δ^{18}O in xylem and soil water[25-28], but little attention has been given to the ERD of field crops[29-31]. Other studies have identified the proportional contributions of water from each soil depth to crops on the basis of isotopic mixing models (e.g., IsoSource[30,31]), Zhang et al.[30] reported that the ERD of winter wheat in the North China Plain was 0-40 cm. However, in contrast to the sampling depths of 100 cm, winter wheat may have root depths of up to 300 cm[32] in the Loess Plateau of China.

In the Loess Plateau of China, winter wheat is one of the most extensively grown crops. Given the inadequate precipitation in this region, the uptake of soil water from the roots is important. However, few studies have investigated the essential ERD of winter wheat on the basis of water stable isotopes technology[32], and even fewer studies have discussed the relationships among the contributions to water uptake of different root depths, root distributions, and soil water content[23,33]. In this research, the ERD, which was measured using the stable isotopes technology, the root distribution, and soil water content were determined in a pot experiment by using PVC tubes. The objectives of this study were: (1) to determine variations in the ERD of winter wheat; and (2) to explain relationships among the contributions to water uptake of different levels of root depth, root distributions and soil water content.

2 Materials and methods

2.1 Experimental site and design

The study was conducted using a pot experiment during the winter wheat growing period. The experiment site is located in the southern region of Shanxi Province (34°48′27″N, 110°41′23″E; 370 m above sea level). The site has a continental temperate monsoon climate with an average annual rainfall of 550 mm, varying within the range of 288-919 mm. More than 70% of the rainfall occurs from June to September. The soil in the study region is loam. The basic characteristics of the soil in the study area after fertilization are listed in Table 1.

Table 1 Basic characteristics of soil in the study area

Depth/cm	Bulk density /g·cm^{-3}	Saturated water content/% (V/V)	Field capacity /% (V/V)	Total nitrogen content/g·kg^{-1}	Available nitrogen content/mg·kg^{-1}	Total phosphorus content/g·kg^{-1}	Total potassium content/g·kg^{-1}	Organic matter /g·kg^{-1}
0-20	1.49	36.73	29.58	1.15	62.90	0.77	19.43	20.20
20-50	1.61	37.35	27.64	0.36	25.16	0.18	17.92	10.00
50-90	1.62	37.34	29.05	0.41	28.76	0.45	17.92	5.69
90-130	1.63	38.40	30.38	0.66	16.18	0.36	19.43	6.47
130-210	1.54	42.84	34.03	0.50	10.78	0.37	19.43	3.53
210-300	1.51	40.92	31.65	1.04	7.19	0.07	17.92	2.94

The winter wheat growth tubes were made of PVC, with a depth of 3 m and an inner diameter of 30 cm. The tubes filled with soil excavated from the study site were sealed at the bottom with plastic film and buried in the soil and the top of each tube was level with the surrounding winter wheat field. The soil samples were compacted every 5 cm with a rammer to ensure the required bulk density in the different soil layers (Table 1). After the experiment, the measured bulk densities of the soil in the tubes demonstrated that no systematic variation existed between the surrounding field and the tubes, thus suggesting that the repacking process disturbed the soil in the tubes to an insignificant extent. Before sowing, the field was fertilized with diammonium phosphate

(75 kg/km^2). At the three-leaf stage, the seedlings inside each tube were thinned to three plants, which was similar to the density in the field experiment. Numerous studies have demonstrated that irrigation could affect root growth directly. However, in this research, we did not treat irrigation as a variable because the objective of the study was to determine variations of the ERD of winter wheat. Thus, to ensure the applicability of the experimental results, an irrigation procedure in accordance with local tradition was applied to the tubes. Winter wheat was irrigated five times (at the wintering, green, jointing, heading, and filling stages). The irrigation time and amount are shown in Figure 1.

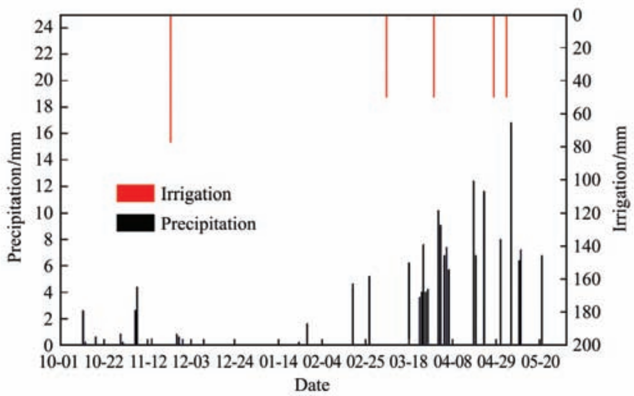


Figure 1 Irrigation and precipitation during the experiment period

2.2 Field sampling and measurements

In each growth period (wintering, green, jointing, heading, filling, and ripening stages), four PVC tubes were pulled out in a completely randomized manner. A total of 24 tubes were collected from 23 October 2014 to 1 June 2015. The tubes were destroyed to recover the plants and soil columns inside each tube. Winter wheat stems at a point far from leaves were collected without the epidermis and placed in airtight vials, which were sealed with parafilm and kept in a cool box with ice. The depth of the corresponding soil columns was based on the MRD of winter wheat at different growth stages at intervals of 10 cm. Some soil samples were collected in the same manner as the stems, but other samples were sealed in bags for analysis of the roots. The stems and soil were sampled at a time chosen when the transportation and evapotranspiration of soil water were low. Subsequently, all the samples were transported to the laboratory and refrigerated at –20°C.

Soil samples were manually removed from the bags in washing cans. The resulting mixture of roots and organic debris was placed in a polythene bag and preserved in a refrigerator until being sorted. After separating the live roots (white or pale brown) from other debris and dark roots, the root length was measured using WinRHIZO software (Regent Instruments Inc., Canada). The RLD at different depths was calculated by dividing the root length by the sampled soil volume (cm/cm^3). To define the vertical distribution of the RLD, we used three parameters that were previously proposed in the literature[2,6,15], namely, the depth where the RLD was greater than 1 cm/cm^3 ($D_{RLD>1}$), the depth where 95% of the roots were located (D_{95}), or half of the MRD (D_{half}). These parameters were deemed to be correlated with plant water use.

During the root sampling process, some soil samples were collected to measure the gravimetric soil water content (SWC) by drying the samples in an oven at 105ºC for 24 h. Subsequently, the soil volumetric water content was calculated by multiplying the corresponding bulk densities. The soil water variation (SWV) with depth was calculated on the basis of the difference in water content between two adjacent sampling times. The weekly soil moisture content was monitored with Diviner 2000 capacitance sensors (Sentek Environmental Technologies, Kent Town, South Australia) to a depth of 160 cm at intervals of 10 cm.

2.3 Isotopic and statistical analysis

Water was extracted from the soil and stem samples by the cryogenic vacuum extraction method at 90°C and 1 Pa. After extraction for approximately 2 h, the extracted water was pipetted from U-tubes into glass vials (2 mL) for isotopic analysis. The δ^2H and δ^{18}O values of water from the soil and stem samples were measured with a Picarro L2130-i analyzer and calculated as follows:

$$\delta^{18}\text{O or } \delta^2 H = (\frac{R_{sample}}{R_{standard}} - 1) \times 1000 \text{‰}$$

where, R_{sample} and $R_{standard}$ are the ratios of the heavier to the lighter isotope of the sample and standard (Vienna Standard Mean Ocean Water, VSMOW), respectively. Organic contaminants in distilled water can affect the results of isotope component analysis of IRIS technology[34]. Therefore, a micro-pyrolysis module (A0214) and ChemCorrect post-processing software were employed to remove the interference of organic material and correct the results. The measured values were calibrated to three standard samples (SLAP2, VSMOW2, and GISP) from the International Atomic Energy Agency. The δ^2H and δ^{18}O values of the water samples were determined with a precision of ±0.1‰ and ±1‰, respectively.

IsoSource software was employed to determine the range of proportional contributions of each water source[32]. In this research, the water sources were defined as the soil waters in layers from the surface roots to the MRD, and were divided into seven or eight groups according to their isotopic composition to represent the potential water sources. Within the IsoSource program, the increments were set at 1% solution and a tolerance level of ±0.2% was counted as feasible solutions.

However, IsoSource does not provide information about the depths of plant water uptake, so we also used another model to obtain more information. The model developed by Romero-Saltos et al.[35] was also used to estimate the mean depth of water uptake by winter wheat. This model was written in MATLAB and could estimate the mean depth of water uptake at a given time, on the basis of the isotopic composition of plant and soil water. Although the model was constructed with two assumptions and one axiom, we do not discuss the exactitude of the assumptions and axiom in this paper. Instead, we simply analyze the solutions for the depth of water uptake obtained by the model.

Data were mapped using the SigmaPlot12.5 and Surfer8.0 programs. SPSS17 software was used for path analysis[36]. A normality test was conducted by Q-Q plots in SPSS17. A logarithmic transformation was required when the distribution was not normal. Differences were considered significant at a level of 0.05.

3 Results and discussion

3.1 Root and soil water content distributions

We used three vertical distribution parameters (Table 2) to summarize the root distribution, instead of the overall root length and depth, because these parameters were deemed to be correlated with plant water use[2,6,15].

As shown in Table 2, $D_{\mathrm{RLD>1}}$ increased during the growth stage, rapidly increased between the jointing and filling stages, and then decreased on ripening. This variation was similar to the results reported by Zhang et al.[14], although the root depth in their research was shallower. A similar situation for D_{95} was also found in this study, because the value of D_{95} was positively correlated with plant transpiration[6]. The highest transpiration rate of winter wheat was observed in the filling stage[30]. However, D_{half} remained constant after the heading stage because the value of D_{half} was associated with the MRD, which already reached up to 3 m in the heading stage. A possible reason for this result was that the tubes still had some influence on crop production, although the tubes were buried in the field to minimize edge effects in the pot experiment. To better explain the observed root depth, future studies should be undertaken to describe the difference between tubes and field conditions.

Table 2 Variation in root distribution parameters in the growth stages

unit: cm

	Wintering	Green	Jointing	Heading	Filling	Ripening
$D_{\mathrm{RLD>1}}$	20	50	110	140	180	110
D_{half}	35	50	100	150	150	150
D_{95}	50	80	130	150	250	210

Figure 2 shows the temporal and spatial distribution of the average SWC in the soil layers from 0 to 160 cm. In a vertical soil profile, the root density usually decreased exponentially with an increase in soil depth. However, the soil moisture gradually increased with an increase in soil depth[32], and decreased as the winter wheat grew[37]. The SWC at the depth of 0-20 cm was variable and ranged from 20% to 40% of the field capacity (FC), which was a consequence of soil surface evaporation and precipitation. At the 40-120 cm depth, the SWC was approximately 70% of the FC and markedly decreased 180-200 d after sowing. This result might be due to the consumption of water by winter wheat during the growth stage. The SWC at greater depths (140-160 cm) was more or less constant.

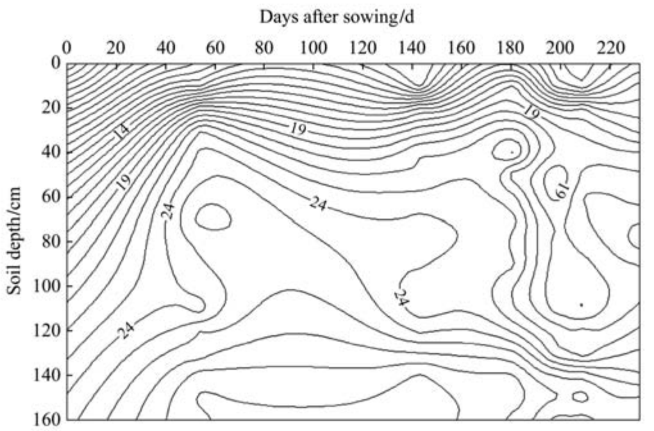
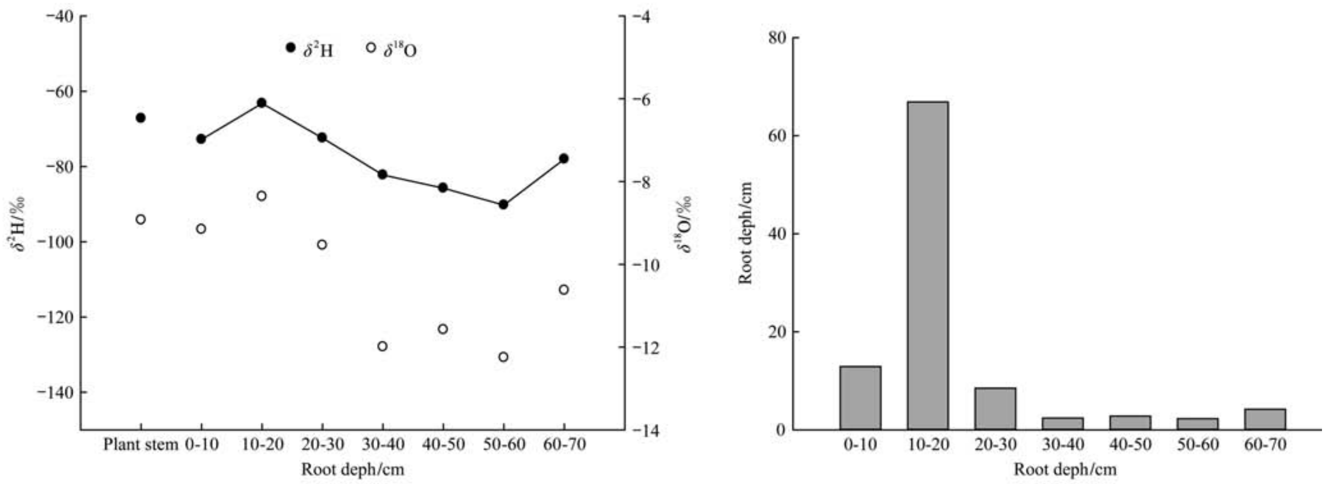


Figure 2 Temporal and spatial distribution of mean soil volumetric water content (V/V %)

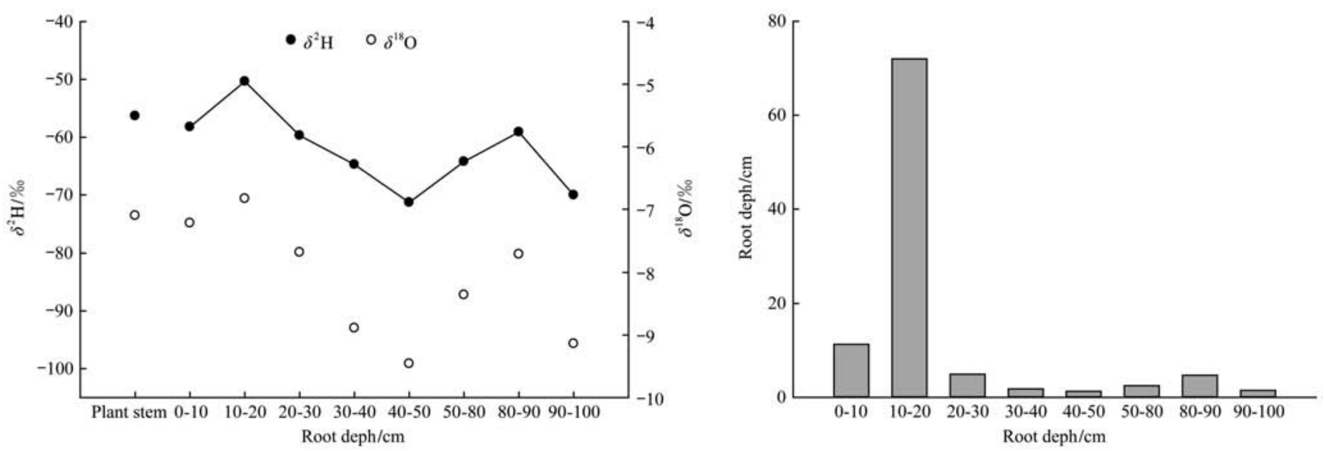
3.2 Effective root depth of winter wheat based on water stable isotopes

As shown in Figure 3 (left panels), the isotopic composition of the soil layers generally varied with changes in root depth. Possible reasons for this result were the influence of precipitation and irrigation water, as well as soil water evaporation and migration[38]. Variations in δ^{18}O and δ^{2}H values with the root profile depth in Figure 3 (left panels) showed a similar trend. Therefore, the use of δ^{18}O was feasible for analyzing the proportional contributions to water uptake of different root depths with the IsoSource model. The changes in ERD over time were indicated by the mean water contributions of different root depths (Figure 3, right panels).

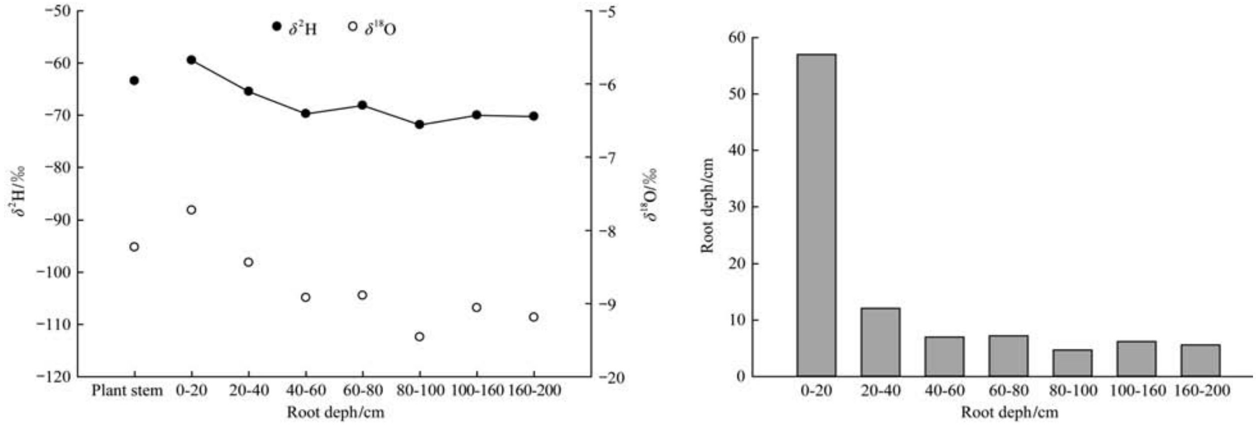
The histograms for all growth stages show that the ERD was 0-20 cm in the wintering and green stages, with water contributions of 79.8% and 83.3%, respectively (Figure 3a and 3b). In the subsequent jointing stage, the ERD increased to 0-40 cm with a contribution of 69.1%, whereas the contribution of depths of 0-20 cm decreased markedly to 57% (Figure 3c). During the heading stage, more deep soil water was utilized by the roots (Figure 3d), and the ERD was concentrated in the ranges of 0-40 cm, 80-100 cm and 160-180 cm. In terms of the filling stage, the ERD range extended to 60-80 cm, 180-200 cm, and 200-220 cm (Figure 3e). For the ripening stage, the ERD returned to 0-40 cm and 80-100 cm (Figure 3f).



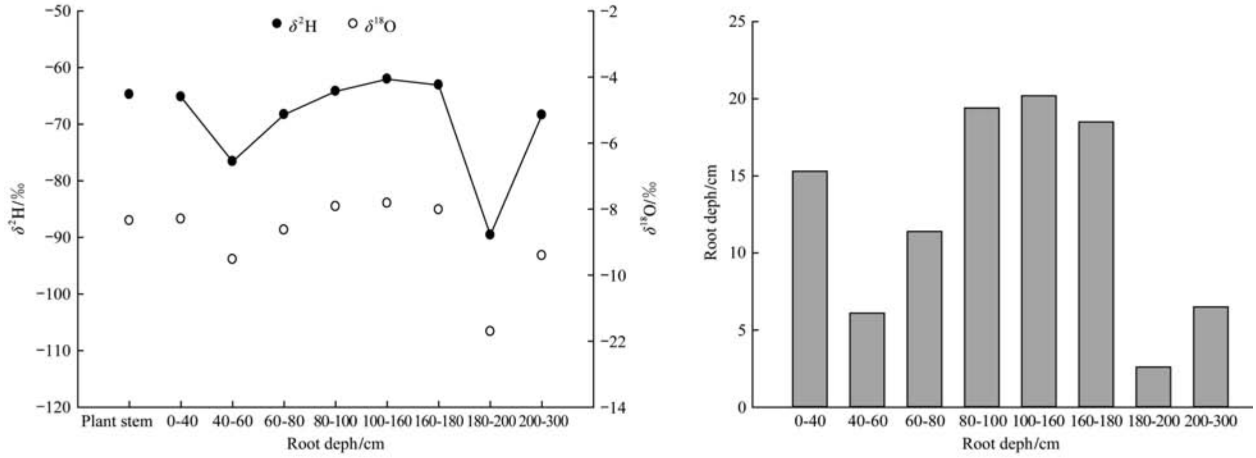
a. Wintering



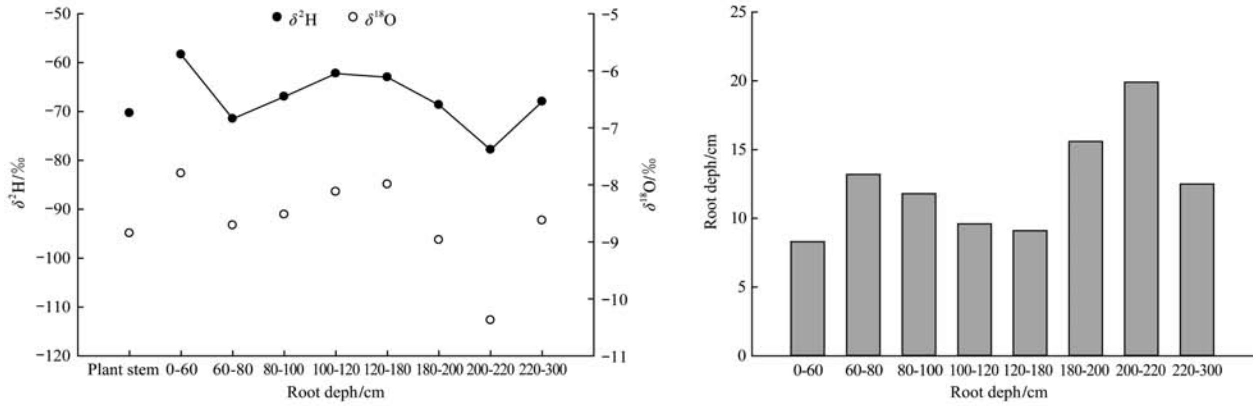
b. Green



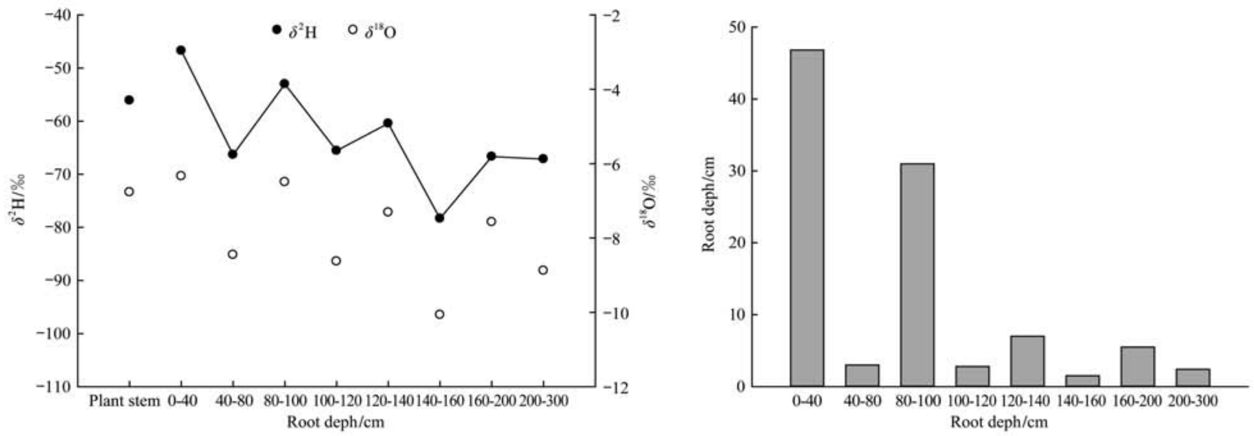
c. Jointing



d. Heading



e. Filling



f. Ripening

Figure 3 Isotopic composition of soil water throughout the root profile and stem water of winter wheat (left panels), and the contributions to water uptake of different root depths (right panels) in all growth stages

The Romero-Saltos model was first used to determine the mean depth of water uptake for winter wheat. According to the Romero-Saltos model, the ERD was 33.81 cm in the jointing stage, increased to 41.94 cm, 87.20 cm and 177.97 cm for the heading stage, and reached its highest values of 8.10 cm and 197.27 cm during the filling stage. However, when the plant was close to ripening, the ERD declined to 44.82 cm.

On the basis of analytical results comparison of the two methods, it was concluded that the ERD gradually increased with the number of days after sowing. The ERD was generally at 0-40 cm during the majority of the growth stages. However, in the heading and filling stages, the ERD could be 60%-75% of the MRD (3 m). This result was not exactly consistent with the results of Zhang et al.[30], who reported an average depth of 40 cm during all growth stages of winter wheat. Nevertheless, Fan et al.[14] observed that effective root zone was 50-100 cm for wheat, and Kirkegaard et al.[39] found that water stored in the range of 1.35-1.85 m became available during the filling of wheat. Zhang et al.[40] revealed that winter wheat obtained its water mainly from the soil at depths of 80 cm and 180 cm during the milk stage. Wang et al.[41] demonstrated that supplemental water in 70% of the soil profile (2 m) after jointing could increase grain yields. Therefore, compared with the traditional irrigation wetting depth of 100 cm[10,30], the irrigation depth could be decreased in the earlier stages and increased during the heading and filling stages to match the ERD of winter wheat.

3.3 Relationships among root length density, soil water variation and the water contributions of different root depths

The aforementioned results showed that the water uptake contributions (WUCs) of different root depths had no significant relationship with the root depth, whereas the SWC reflected an increase in the soil profile. However, as shown in Table 3, when considering the SWV and RLD separately, the WUCs of different root depths were correlated with the SWV (r=0.395, p<0.05) and RLD (r=0.368, p<0.05). A similar relationship was also found by Liu et al.[23], who analyzed the relationship between root biomass and water uptake patterns, although they showed the SWC profile, which was also correlated with the WUCs of different root depths. The difference might be due to a higher water content in the upper layers and the presence of the roots of the shrub in the whole soil profile, but the roots of winter wheat gradually increased during the growth stages.

Table 3 Correlation coefficients for RLD, SWV and WUCs

	SWV	RLD	WUCs
SWV	1	0.252	0.395
RLD		1	0.368
WUCs			1

On the basis of path analysis (Table 4), the SWV was the main deciding variable for the WUCs of different root depths, with a higher direct function and decisive coefficient than the RLD. Furthermore, the SWV would indirectly affect the WUC via RLD, of which the indirect function was 0.072. Therefore, the interaction between the SWV and RLD combined the effects of relationships between the water uptake patterns of winter wheat and its root distribution and soil water conditions.

Table 4 Path analysis of the water uptake contributions of different root depths

Independent	Direct	Indirect	Decisive coefficient
SWV	0.323	0.072	0.151
RLD	0.287	0.081	0.128

However, the decisive coefficient was low, which demonstrated that the root distribution and SWC profile did not always follow the same trends as the water uptake. Other physiological properties and factors need to be considered, such as the root activity, neighboring plants, nutrient levels, irrigation method and climatic conditions[8,9]. These studies indicate that the stable isotopes technology is a new way of representing root water uptake, and thus, demonstrates an integrated response of root systems to different soil water profiles with different isotopic signatures.

4 Conclusions

This study had two main objectives. Firstly, this work aimed to reveal variations in the ERD of winter wheat in its various growth stages by using IsoSource and the Romero-Saltos model. The ERD range generally increased from the wintering stage to the filling stage and then decreased in the ripening stage. A root depth of

0-40 cm in the vertical profile was essential for water uptake by winter wheat, whereas the deep roots (60%-75% of the MRD) seemed more important in the heading and filling stages. Secondly, this study used correlation and path analysis to show that the contributions to water uptake of different root depths matched the root distribution or SWV, and that the SWV was the main deciding variable for the WUCs of different root depths. Determining the ERD of winter wheat and its relationship to the root distribution and soil water in the Loess Plateau of China is critical for understanding the water use patterns of plants and guiding the management of water-saving irrigation.

Acknowledgements

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