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Exploring the differences of moisture traceability methods based on MixSIAR model under different nitrogen applications of wheat in the Arid Region of Northwest China

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A R T I C L E I N F O

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A B S T R A C T

The Bayesian stable isotope mixing (MixSIAR) model was widely used in water source tracing. However, double isotope and single isotope calculated by the MixSIAR model led to different results in terms of previous studies. The effect of different nitrogen treatments on the water traceability results of wheat crops is still unclear. This study investigated the wheat root water uptake patterns at different nitrogen application gradients, as well as at different isotopes in the MixSIAR model. The results showed the main soil water uptake layer was relative constant at the treatment of 15% nitrogen reduction, and the water source was mainly from 0–10 cm soil layer from jointing to harvest. The main soil water uptake layer significantly varied at the treatment of 30% nitrogen reduction, which yielded the highest. 45% reduction of nitrogen treatment showed the deepest soil water uptake absorption on average with the whole wheat growth period. The consistency of the results between the two single isotope methods was better in the early stage of wheat and worse in the later stage. The inconsistency of root water uptake distribution results between the two single isotope methods might be influenced by the water transport mechanism of wheat stem and sampling errors. The double isotope method had the lowest uncertainty, but it might amplify the error of the sampling process. The uncertainty of the single hydrogen isotope method was less than single oxygen isotope method. This study provided a new evaluation of hydrogen-oxygen stable isotope traceability methods for wheat under different nitrogen treatments, and gave more ideas and insights for subsequent crop water traceability by the MixSIAR method.

1. Introduction

The water resource shortage has been one of the main limiting factors in increasing grain production in northwest China (Kang et al., 2017). With the increasing focus on water cycle systems in cropland, assessing crop water sources has been a hot issue in agricultural water resources research (Penna et al., 2020). Accurately quantifying the water sources of crops could help us understand and optimize irrigation methods, leading to higher crop water productivity. Meanwhile, fertilization is an important agronomic measure to motivate crop growth, the increase in nutrients regulates the growth rate of the crop and indirectly changes the water consumption of the crop (Zhang et al., 2020). Leaving aside the potential harm of further environmental pollution and economic loss (Shao et al., 2014; Zhang et al., 2015; Li et al., 2022), the relationship between nitrogen and crop water use pattern was still not clear. Some study already proved that the availability of soil water was an important factor for soil nutrient content (R. Wang et al., 2014; C. Wang et al., 2014), and controlled the loss of nutrients (Bristow et al., 2020; Li et al., 2023), especially under different irrigation method (Sepaskhah and Tafteh, 2012) and irrigation quantity (Pandey et al., 2000). So far, the effect of nitrogen treatment on yield and crop traits has been under more concentrations (Sandhu et al., 2019; Liu et al., 2020; Xu et al., 2020), yet few papers reported how different nitrogen treatments affect crop water use pattern. As the dual impacts of soil water and nitrogen lead to various crop growth patterns, figuring out the crop water use pattern is of great impcortance in formulate irrigation and

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fertilization strategies.

Researchers usually explore crop water requirements by detecting root status (Fan et al., 2017). It was reported that both root length density and dry root matter had a significant correlation with the water uptake proportion (Zhao et al., 2018; Liu et al., 2021). Nitrogen influences crop growth, and changes the root character, so that the water sources of crops might changes under different nitrogen conditions (Ma and Song, 2018; Gao et al., 2022). Therefore, changing the nitrogen application to regulate root growth is an effective way to alter the soil water use pattern of crops without considering the impact of changes in water content. However, root systems studies were limited by survey methods (Thorup-Kristensen et al., 2020), which cannot reflect the temporal and spatial variation of the selective used of soil water by crop root zone accurately (Freyberg et al., 2020).

The stable isotope method provides a powerful tool for quantifying root water sources. Over the past decade, it has been applied in agricultural systems to compare plant water use under different tillage practices (Liu et al., 2020), under different fertilization treatments (Ma and Song, 2016; Gao et al., 2022), and under different irrigation methods (Wu et al., 2016). Water tracing based on the principle of root water uptake with no isotopic fractionation helped people understand the important processes of plant water selective used in the terrestrial water cycle (Ehleringer and Dawson, 1992; Sprenger et al., 2016), especially in agricultural soils with variable treatments (Penna et al., 2020). Due to external factor changes (such as precipitation and temperature), stable isotope in different soil layer typically exhibits a staircase-like distribution (Wu et al., 2016), which also provides the possibility of using stable hydrogen and oxygen isotopes for water source tracing. Two main methods were usually used for crop water source identification: the direct comparison method and the model calculation method. The direct comparison method typically compares the soil profile distribution of a single hydrogen or oxygen isotope with that of plant xylem water, assuming that crop water only comes from a single soil layer (Bing et al., 2016). Although this method is simple and fast, it ignored the diversity of crop water uptake, and the tracing results of the two isotopes were not completely consistent (Wang et al., 2019). The model calculation method of linear mixing models including IsoSource (Phillips and Gregg, 2003), Bayesian hybrid model MixSIR (Moore and Semmens, 2008), Stable Isotope Analysis in R (SIAR) (Parnell et al., 2010), and the Bayesian stable isotope mixing (MixSIAR) model (Stock et al., 2018) provide frameworks for calculating the plant root water uptake distribution, that have been commonly used in recent 20 years. Among the linear mixing models above, the MixSIAR model is more accurate and reliable due to the application of multiple prior information (Wang et al., 2019).

Among all of the isotope-based root water uptake studies in recent decades, about 61% focused on a single isotope (δ^2H or δ^{18}O) framework for water source tracing (Huo et al., 2020; Xu et al., 2021), while only 25% of research was based on a double isotope (δ^2H and δ^{18}O) framework (Rothfuss and Javaux, 2017). Even though the distribution of the two single isotopes had a strong correlation, it still brought about differences in the water source tracing results (Wang et al., 2019). Previous studies showed that differences between hydrogen and oxygen isotopes might arise in water allocation calculations in tree species (Yang et al., 2015) or comparing different modeling approaches (Wang et al., 2019), but few moistures traceability research was conducted under different nitrogen application treatments in wheat crops under field conditions. Therefore, more research is required to compare the differences between single and double isotopes when using water traceability model to better compare between different studies. Especially for field crops, environmental changes lead to large individual differences (Brunel-Saldias et al., 2020). So that more studies are needed to evaluation for affecting the accuracy of traceability results. In this study, we aim to address the following questions by conducting irrigation experiments on spring wheat under different nitrogen treatments and using the MixSIAR model as a representative: (1) comparing the water sources of wheat under different nitrogen treatments, (2) explaining the relationship between the nitrogen supply, the root water uptake patterns, and the yield, and (3) comparing the root water uptake proportion differences under different isotope methods.

2. Material and methods

2.1. Study site

The field experiment was conducted in 2022 at the Shiyanghe Experimental Station of China Agricultural University, located in Wuwei City, Gansu Province, China (37°52′N, 102°51′E, altitude 1580 m). The station is in a typical continental temperate climate with an average annual rainfall of around 164 mm, an annual evaporation of over 2000 mm, an average annual temperature of 8 ℃, over 3000 h of annual sunshine, and an average groundwater level below 40 m. Therefore, the impact of groundwater was not considered in this study. The meteorological conditions during the study year were shown in Fig. 2.

2.2. Experimental design

The planting of spring wheat experiment was set on 03/22/2022 and harvested on 07/19/2022. The total irrigation amount during the growth period was 287 mm, and the nitrogen fertilization time and amount were shown in Table 1. Five different nitrogen application treatments were set up. N0: no nitrogen application, N1: normal nitrogen reduction, N2: nitrogen reduction by 15% (191.25 kg/hm^2) based on the normal nitrogen reduction, N3: nitrogen reduction by 30% (157.50 kg/hm^2) based on the normal nitrogen reduction, and N4: nitrogen reduction by 45% (123.75 kg/hm^2) based on the normal nitrogen reduction. And the normal nitrogen reduction was a total pure N supply of 225 kg/hm^2. The pure P_2O_5 was applied as base fertilizer, whose supply was 150 kg/hm^2. Each plot was 8.6 m long and 6 m wide, with a total area of 51.6 m^2. 225 kg/hm^2 nitrogen application is the common practice of local spring wheat planting (Sun et al., 2021; Kamran et al., 2023), and nitrogen reduction between 0 to 45% includes the best result of all the reduction treatments (Chen et al., 2018; Kamran et al., 2023). As a result, five different nitrogen application treatments above were set up. Each treatment had three replicate samples under different replicate plot, and each replicate included one xylem water sample and six soil water samples. Five different nitrogen application treatments were included. Finally, the wheat yields were 1414.7 kg/hm^2, 4379.8 kg/hm^2, 5478.0 kg/hm^2, 6472.9 kg/hm^2, and 5374.7 kg/hm^2 under N0, N1, N2, N3, and N4 treatments, respectively.

2.2.1. Isotopic sampling and measurements

Xylem samples of wheat and soil samples were simultaneously collected on the jointing stage on 04/26, the booting stage on 05/19, the bloom stage on 06/06, and the filling stage on 06/23 of 2022. To avoid the influence of drastic fluctuations in soil moisture, the sampling date was at least three days after water input events such as precipitations or irrigations. In case to avoid stable isotope fractionation, the xylem sampling sites were restricted to the white unwooded area of the wheat stem nearby soil. Soil samples were obtained using a soil auger at depths of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–120 cm near the stem. The collected samples were sealed in Ziplock bags and stored at a − 20 ℃ fridge until cryogenic extraction. Precipitations were collected using a measuring cup. After the rainfall event, the rainfall samples were stored in centrifuge tubes and sealed with sealing film until further analysis. Irrigation samples were collected during each irrigation event. In total, 15 xylem samples, 90 soil samples, 23 precipitation samples, and 7 irrigation samples were collected in this study.

The xylem and soil samples were extracted from liquid water using an automatic cryogenic extraction system (Li-2100, LICA United Technology Limited, Beijing, China). To ensure full extraction, the extraction time was set to 3 h, the extraction temperature was set to 198 °C, and the

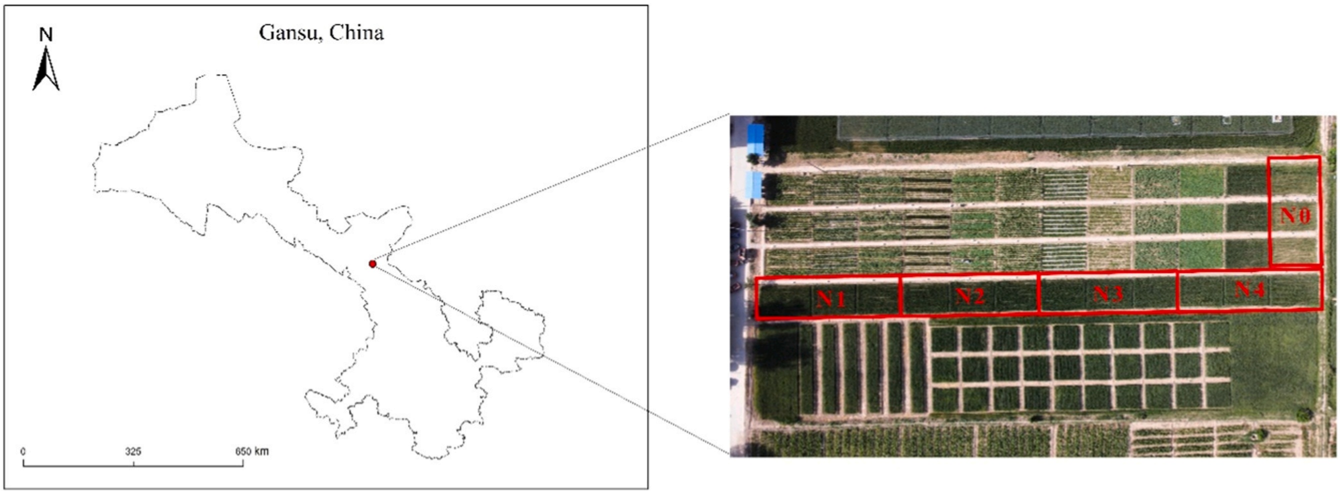

Fig. 1. Local of the experiment station site on the left and different field experiment treatments distribution on the right.

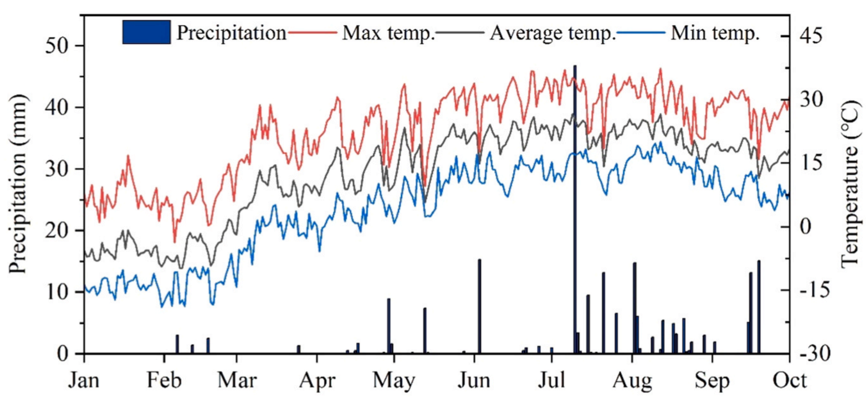

Fig. 2. Precipitation and temperature from January to October of 2022.

Table 1
Fertilization and irrigation time schedule ("mm/dd" stands for the date of the implement on year 2022) under N0, N1, N2, N3, and N4 treatment, respectively.

Treatments	N0	N1	N2	N3	N4
Fertilization on 03/20 (kg/hm^2)	0	135	115	94	74.5
Fertilization on 05/02 (kg/hm^2)	0	90	76.5	63.5	50
Irrigation on 05/02 (mm)	60	60	60	60	60
Irrigation on 05/20 (mm)	38	38	38	38	38
Irrigation on 05/31 (mm)	75	75	75	75	75
Irrigation on 06/16 (mm)	75	75	75	75	75
Irrigation on 06/30 (mm)	39	39	39	39	39

condensation temperature was set to − 95 °C. The extraction rate could reach over 98% (Wang et al., 2017).

All water samples (xylem, soil, precipitation, and irrigation) were measured using a high-precision stable hydrogen and oxygen isotope analyzer (PICARRO L2130-i, Picarro, USA). To minimize measurement errors and eliminate instrument uncertainty, each sample was measured six times, and the average of the last three results was taken as the final values. The stable isotope values were calculated using the Vienna Standard Mean Ocean Water (VSMOW) as a reference standard.

$$\delta^{18}O\left(\delta^{2}H\right) = \left[\frac{R_{Sample}}{R_{S\tan dard}}\right] \times 1000, \quad (1)$$

where R_{Sample} and $R_{Standard}$ representative atomic abundance of average standard seawater in δ^{18}O (δ^{2}H) (‰). After obtained the isotope values of each source of water, we were able to obtain the local meteoric water line (LMWL) and local irrigation water line (IWL) in terms of the relationship between δ^{18}O and δ^{2}H of precipitation and irrigation water, respectively.

2.2.2. MixSIAR model setup

For the convenience of calculation, the six soil layers in 2.2.1. were classified into four soil layers. According to the deuterium excess value (D-excess = δ^{2}H - (8δ^{18}O + 10)) of stable hydrogen and oxygen isotopes, indicated that the evaporation intensity, the new soil layers include: (1) 0–10 cm, which shows a relatively large variation due to seasonal rainfall and irrigation and other input water sources and environmental changes; (2) 10–20 cm, which is still affected by evaporation and input water sources, but with a smaller variation than the surface layer; (3) 20–60 cm, which is less affected by evaporation and more susceptible to the influence of water input; and (4) 60–120 cm, which belongs to the deep soil layer with relatively stable values of stable isotopes. The isotopic values of the merged soil layers were calculated using the isotope mass conservation method, with consideration of the soil volumetric water content.

$$\frac{\delta_{S1}\theta_{S1} + \delta_{S2}\theta_{S2}}{\theta_{S1} + \theta_{S2}} = \delta_{S}, \quad (2)$$

where δ_{S1} and δ_{S2} represent the hydrogen or oxygen isotope values measured in soil layer 1 and soil layer 2, θ_{S1} and θ_{S2} represent the soil volume moisture in soil layer 1 and soil layer 2, and δ_{S} represents the hydrogen or oxygen isotope value of the combined soil layer.

The local underground water (below 40 m) cannot be used directly by crops, and no surface runoff was observed in the study. Thus, soil water was considered as the only water source for wheat. In this study, wheat xylem water was used as the source input for the MixSIAR model (version 3.1.12) with three different model application methods: (1) Single $\delta^{18}O$ method (SOM), which only input the $\delta^{18}O$ values of wheat xylem water and soil water; (2) Single $\delta^{2}H$ method (SHM), which only input the $\delta^{2}H$ values of wheat xylem water and soil water; (3) Double isotope method (DIM), which input both $\delta^{18}O$ and $\delta^{2}H$ values of wheat xylem water and soil water. The MixSIAR model was run 45 times, including 15 times each for SOM, SHM, and DIM, respectively. As the individual 15 times' running contained three replicates of the five nitrogen treatments, the three replicates were averaged to the final output of five nitrogen treatments. The standard deviations of the three replicates were also output. Since the fractionation effect during the crop water uptake process was not considered, the discrimination values of $\delta^{18}O$ and $\delta^{2}H$ were set to 0. The Markov Chain Monte Carlo (MCMC) run length was set to "long", and no prior information was set in the model. Gelman-Rubin and Geweke diagnostic tests were used to determine whether the model converged, and the error structure was set to "Residual only".

2.2.3. Validation of different methods

The three different model application methods were validated by reconstructing the isotopic composition of the source water (Wang et al., 2019). The predicted isotopic composition of xylem water (δ_i) under each method was obtained as follows:

$$\delta_i = \sum_{j=1}^{k} f_j \delta_j, \tag{3}$$

where f_j was the mean contribution proportion of soil water calculated by the model. δ_j is the water isotope value corresponding to the layer of soil ($\delta^{18}O$ or $\delta^{2}H$).

To compare the uncertainties of the MixSIAR model among all three methods, the mean percentage error (MPE) was applied:

$$\text{MPE} = \left| \frac{1}{n} \sum_{i=1}^{n} \frac{\delta_i - O_i}{O_i} \right|, \tag{4}$$

where o_i was the measured isotopic value of wheats xylem water ($\delta^{18}O$ or $\delta^{2}H$), n = 3 represented three independent replicate nitrogen treatments. MPE ranges from 0 to 1. When MPE is closer to 0, it indicates higher simulation accuracy of the model (Li and Heap, 2011). In this study, we calculated the MPE of SOM (MPE_{SOM}), the MPE of SHM (MPE_{SHM}), and the MPE of DIM (MPE_{DIM}), respectively. MPE_{DIM} included the $\delta^{18}O$ of MPE_{DIM} ($MPE_{DIM}(\delta^{18}O)$) and the $\delta^{2}H$ of MPE_{DIM}

Table 2
The isotope compositions ($\delta^{18}O$ and $\delta^{2}H$) of precipitation, irrigation, soil water (different depth), and xylem water under different treatment (N0 to N4).

Treatment	Samples	Soil Samples'	$\delta^{18}O$ (‰)				$\delta^{2}H$ (‰)			
		Depth (cm)	Max	Min	Average	SD	Max	Min	Average	SD
-	Precipitation	-	5.03	-57.79	-3.40	4.13	36.88	-57.79	-12.68	27.28
-	Irrigation	-	-9.50	-9.65	-9.57	0.05	-59.31	-60.06	-59.80	0.25
N0	Soil water	0-10	-2.04	-8.54	-5.57	2.00	-22.56	-56.97	-44.35	9.79
		10-20	-6.42	-9.87	-8.64	1.02	-47.36	-61.29	-57.18	3.81
		20-40	-8.34	-10.38	-9.42	0.54	-55.01	-63.45	-59.76	2.34
		40-60	-9.16	-10.14	-9.59	0.28	-58.01	-62.84	-60.56	1.35
		60-80	-8.97	-10.33	-9.59	0.35	-59.36	-63.52	-60.92	1.32
		80-120	-6.97	-10.80	-9.34	0.87	-50.97	-63.45	-59.50	3.04
	Xylem water	-	-3.98	-7.46	-5.48	1.09	-38.23	-56.55	-45.38	4.55
N1	Soil water	0-10	-2.49	-7.62	-4.96	1.47	-22.23	-52.57	-38.86	10.28
		10-20	-6.68	-10.70	-8.67	1.15	-55.23	-62.32	-58.40	1.80
		20-40	-8.11	-10.23	-9.21	0.61	-56.33	-63.12	-59.71	2.01
		40-60	-7.62	-10.02	-8.78	0.67	-52.28	-64.56	-58.80	2.73
		60-80	-8.04	-9.86	-8.93	0.47	-56.49	-65.60	-59.56	2.37
		80-120	-8.19	-9.77	-9.05	0.46	-56.12	-63.63	-59.66	2.36
	Xylem water	-	-3.82	-7.82	-6.48	1.05	-41.28	-53.86	-48.18	3.62
N2	Soil water	0-10	-2.47	-6.55	-4.99	1.41	-25.70	-48.17	-39.05	7.83
		10-20	-4.93	-10.60	-8.75	1.71	-51.83	-65.27	-58.81	5.01
		20-40	-8.45	-11.17	-9.78	0.79	-57.89	-67.02	-62.88	2.53
		40-60	-8.97	-10.98	-9.79	0.55	-58.41	-66.77	-62.86	2.31
		60-80	-8.99	-10.49	-9.71	0.43	-58.87	-65.05	-61.67	1.72
		80-120	-8.94	-10.16	-9.75	0.34	-58.40	-66.58	-62.24	2.41
	Xylem water	-	-4.70	-7.25	-6.44	0.82	-45.22	-56.66	-52.20	3.16
N3	Soil water	0-10	-0.92	-7.01	-4.61	1.81	-22.89	-49.85	-36.77	9.09
		10-20	-6.09	-10.19	-8.82	1.27	-49.38	-62.94	-59.11	4.28
		20-40	-8.11	-10.62	-9.52	0.63	-57.94	-64.87	-61.16	2.22
		40-60	-8.86	-10.72	-9.55	0.56	-58.30	-64.70	-61.24	2.18
		60-80	-8.84	-10.61	-9.52	0.49	-58.83	-66.70	-60.88	2.01
		80-120	-8.93	-10.11	-9.57	0.38	-57.81	-63.96	-61.16	1.91
	Xylem water	-	-5.79	-7.99	-6.99	0.80	-48.79	-56.10	-52.72	2.60
N4	Soil water	0-10	4.58	-9.19	-5.17	3.21	-26.36	-59.31	-40.62	9.51
		10-20	-0.49	-10.44	-8.40	2.50	-42.49	-65.24	-58.56	5.55
		20-40	-7.91	-9.98	-9.31	0.60	-58.88	-64.60	-60.72	1.54
		40-60	-8.56	-10.17	-9.56	0.47	-58.32	-63.54	-61.27	1.71
		60-80	-8.83	-10.13	-9.63	0.39	-58.29	-62.92	-60.90	1.40
		80-120	-9.19	-10.29	-9.66	0.31	-59.38	-63.25	-61.16	1.26
	Xylem water	-	-5.34	-8.75	-7.23	0.97	-45.19	-57.60	-52.12	3.73
Total	Soil water	0-10	4.58	-9.19	-5.06	2.11	-22.23	-59.31	-39.93	9.67
		10-20	-0.49	-10.70	-8.66	1.63	-42.49	-65.27	-58.41	4.34
		20-40	-7.91	-11.17	-9.45	0.67	-55.01	-67.02	-60.85	2.44
		40-60	-8.56	-10.98	-9.45	0.63	-52.28	-66.77	-60.95	2.48
		60-80	-8.83	-10.61	-9.48	0.51	-56.49	-66.70	-60.78	1.93
		80-120	-6.97	-10.80	-9.47	0.57	-50.97	-66.58	-60.74	2.50
	Xylem water	-	-3.98	-8.75	-6.52	1.13	-38.23	-57.60	-50.12	4.60

($MPE_{DIM}(\delta^2H)$).

3. Results

3.1. Stable isotope distribution of soil and wheat xylem water under different treatment

As was shown in Table 2, the $\delta^{18}O$ range of irrigation water in the area was − 9.65‰ to − 9.50‰ ($n = 7$) with an average (± 1 SD) of − 9.57‰ (± 0.05), while the δ^2H range was − 60.06‰ to − 59.31‰ ($n = 7$) with an average of − 59.80‰ (± 0.25). The $\delta^{18}O$ range of precipitation was − 10.37‰ to 5.03‰ ($n = 23$) with an average of − 3.04‰ (± 4.13), and the δ^2H range was − 57.79‰ to 36.88‰ ($n = 23$) with an average of − 12.68‰ (± 27.28). The soil water isotope variability in the 0–10 cm layer was significantly greater than that of the deeper soil layers. In terms of the relationship between $\delta^{18}O$ and δ^2H of soil water and xylem water (Fig. 3), the soil water in the 60–120 cm layer was significantly influenced by irrigation water in all treatments. It was alongside the irrigation water line (IWL: $\delta^2H = 3.63\delta^{18}O - 25.04$, $R^2 = 0.48$, $p = 0.084$) yet off from the local meteoric water line (LMWL:

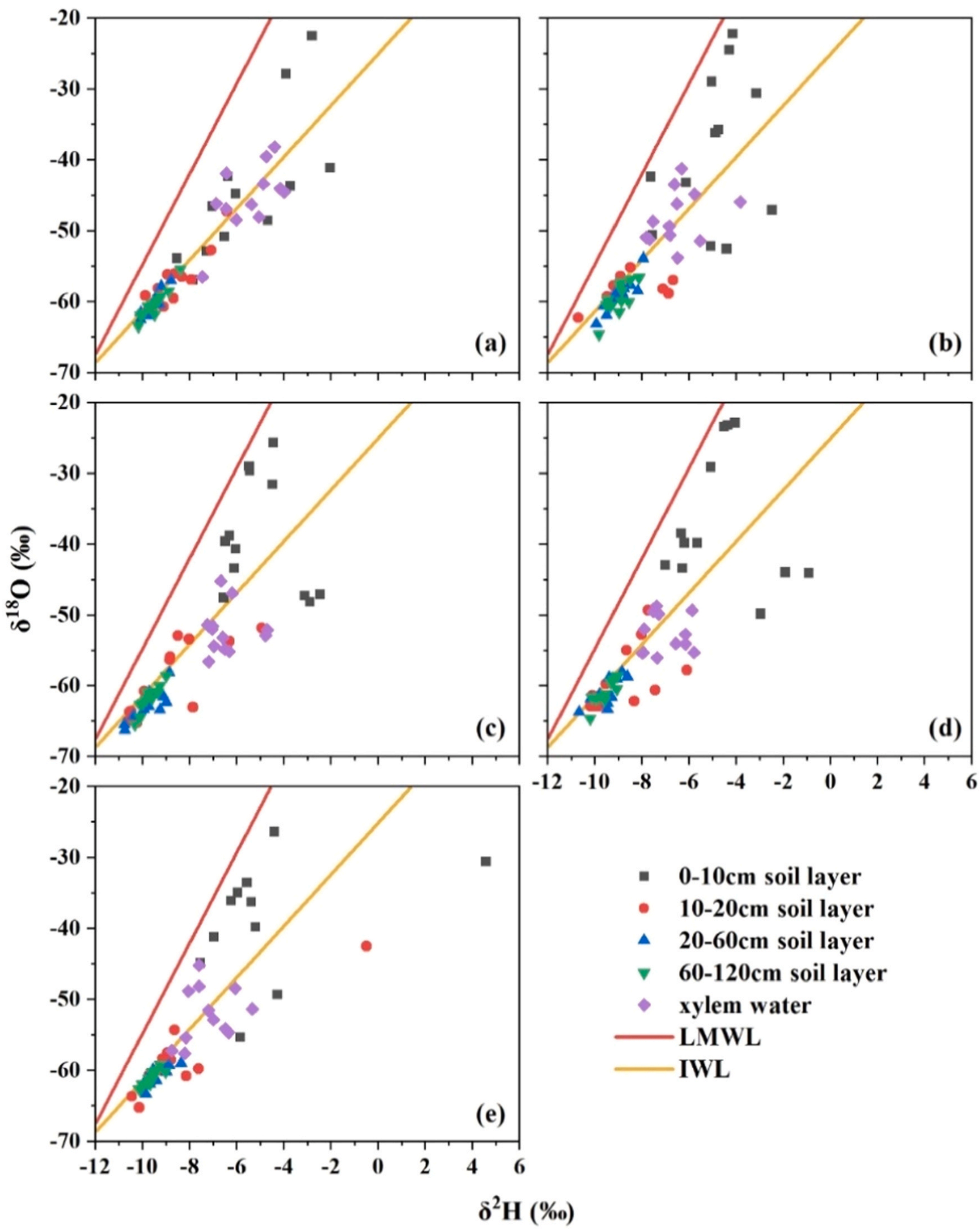

Fig. 3. The relationship between $\delta^{18}O$ and δ^2H of different soil layer and xylem water under (a) N0 treatment, (b) N1 treatment, (c) N2 treatment, (d) N3 treatment, and (e) N4 treatment, respectively. Red line stands for the local meteoric water line (LMWL: $\delta^2H=6.37\delta^{18}O+9.00$), yellow line stands for irrigation water line (IWL: $\delta^2H = 3.63\delta^{18}O-25.04$), respectively.

$\delta^2H = 6.37\delta^{18}O + 9.00$, $R^2 = 0.93$, $p < 0.001$). In 2022, the precipitation during the wheat growing season was only 102.9 mm, occupying 47.77% of the total precipitation from January to October 2022 (215.4 mm). The strong soil water evaporation led to the down deviation of soil and xylem water isotope values from the LMWL. Fig. 3 showed that the surface soil had a greater impact on the water use of wheat crops in the 0–10 cm and 10–20 cm soil layers due to the large variation in isotopic values under double isotopic conditions. Under different nitrogen treatments, except for the significant difference in the oxygen isotopic values of the soil water in the 60–120 cm layer between the N1 and N4 treatments ($p < 0.05$), there was no significant difference between the isotopic values of soil water in other layers among different treatments.

The overall $\delta^{18}O$ wheat xylem water ranged from − 8.75‰ to − 3.98‰ ($n = 60$), with a mean value (± 1 SD) of − 6.52‰ (± 1.13). The overall $\delta^{18}O$ wheat xylem water ranged from − 57.60‰ to − 38.23‰ ($n = 60$), with a mean value (± 1 SD) of − 50.12‰ (± 4.6) (Table 2). Significant differences in the oxygen isotopes of crop xylem water were observed between the N0 and N4 treatments ($p < 0.05$), with mean values (± 1 SD) of N0: − 5.48‰ ± 1.09‰, and N4: − 7.23‰ ± 0.97‰. For the hydrogen isotopes of wheat xylem water, significant differences were found between the N0 treatment and the N2, N3, and N4 treatments ($p < 0.05$), and no significant differences between the other treatments ($p > 0.05$).

3.2. Soil water contribution proportions

As was shown in Fig. 4, the contribution of water from different soil layers under different treatments on different sampling dates was obtained. As for the different isotope methods, the SHM method had 2.1% and 4.8% less 0–10 cm layer soil water usage proportion compared to the SOM method and DIM method, respectively. The SOM method reached a maximum of 33.3% contribution proportion on 05/19 and then slightly decreased to 32.2% contribution proportion on 06/23 in the 0–10 cm soil layer. The SHM method reached a minimum of 27.6% contribution proportion on 06/06 and then slightly increased to 28.4% contribution proportion on 06/23. During this period, the water distribution proportion of wheat in each soil layer was similar. The average distribution proportion results of the double isotope method were different from the two single isotope methods (SHM and SOM). On 04/26, the contribution proportions of the 0–10 cm and 10–20 cm soil layers in the DIM method were 37.2% and 23.3%, respectively. On 05/19, the contribution proportions of the 0–10 cm and 10–20 cm soil layers using the double isotope method were 37.9% and 20.6%, respectively. On 06/06 and 06/23, the water source distributions shifted to the 20–60 cm and 60–120 cm soil layers, and the crop water use trend changed from 0–10 cm to 20–120 cm soil layers.

Here we evaluated the mean contribution proportions by averaging the contribution proportions on all three methods. The 0–10 cm soil

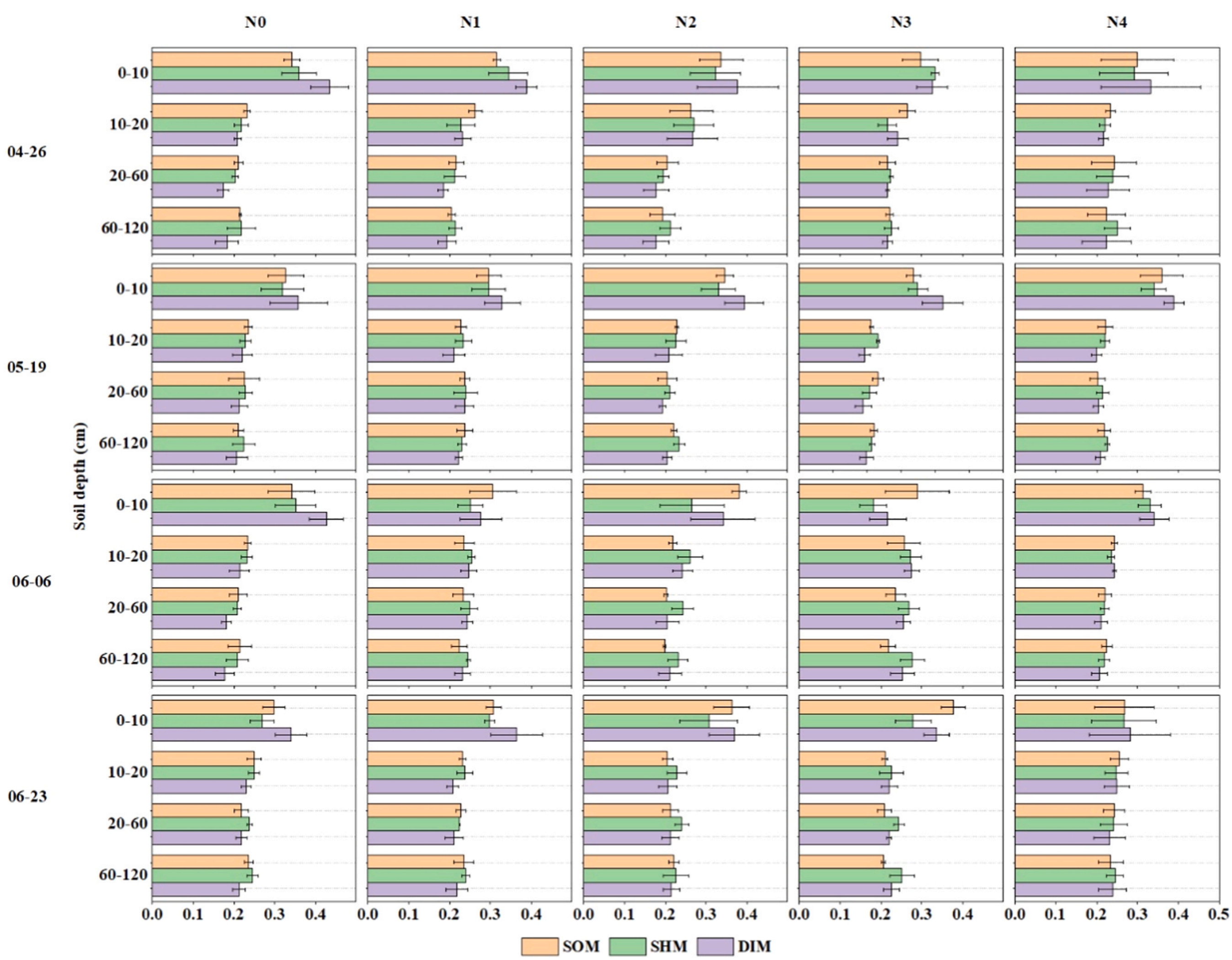

Fig. 4. Soil water contribution proportions under five nitrogen treatments in four days calculated by the Bayesian stable isotope mixing (MixSIAR) model. Three methods were applied: use $\delta^{18}O$ only (SOM), use δ^2H only (SHM) and with two isotope method (DIM). Error bar (± 1 SD) showed the difference between three repetitions of each treatment ($n = 3$).

layer mean contribution proportions were greater than that of other layers, but the contribution ratios were various under different nitrogen treatments. The soil water uptake pattern showed differences between different nitrogen treatments. The mean contribution ratios of the 0–10 cm soil layer from the N0 to N4 treatments were 34.7%, 31.4%, 34.4%, 31.2%, and 31.7%, respectively. This showed the N3 treatment absorbed more soil water from 10–120 cm soil layer than other treatments. The mean contribution ratios of the 10–20 cm, 20–60 cm, and 60–120 cm soil layers were relatively evenly distributed in all treatments (Table 2). On 04/26, the water source distribution among the five nitrogen application treatments was similar, and the mean distribution proportions from the 0–10 cm soil layer among the N0 to N4 treatments were 37.9%, 34.9%, 34.6%, 31.9%, and 30.8%, respectively. The mean contribution ratio of water sources from the 20–60 cm and 60–120 cm soil layers was similar, with an average difference of less than 1%. The water uptake pattern of wheat was affected to different extents by time and nitrogen treatments. During the time series from 04/26 to 06/23, the variability sequence of mean contribution ratios in 0–10 cm soil layer was N3 > N4 > N0 > N1 > N2.

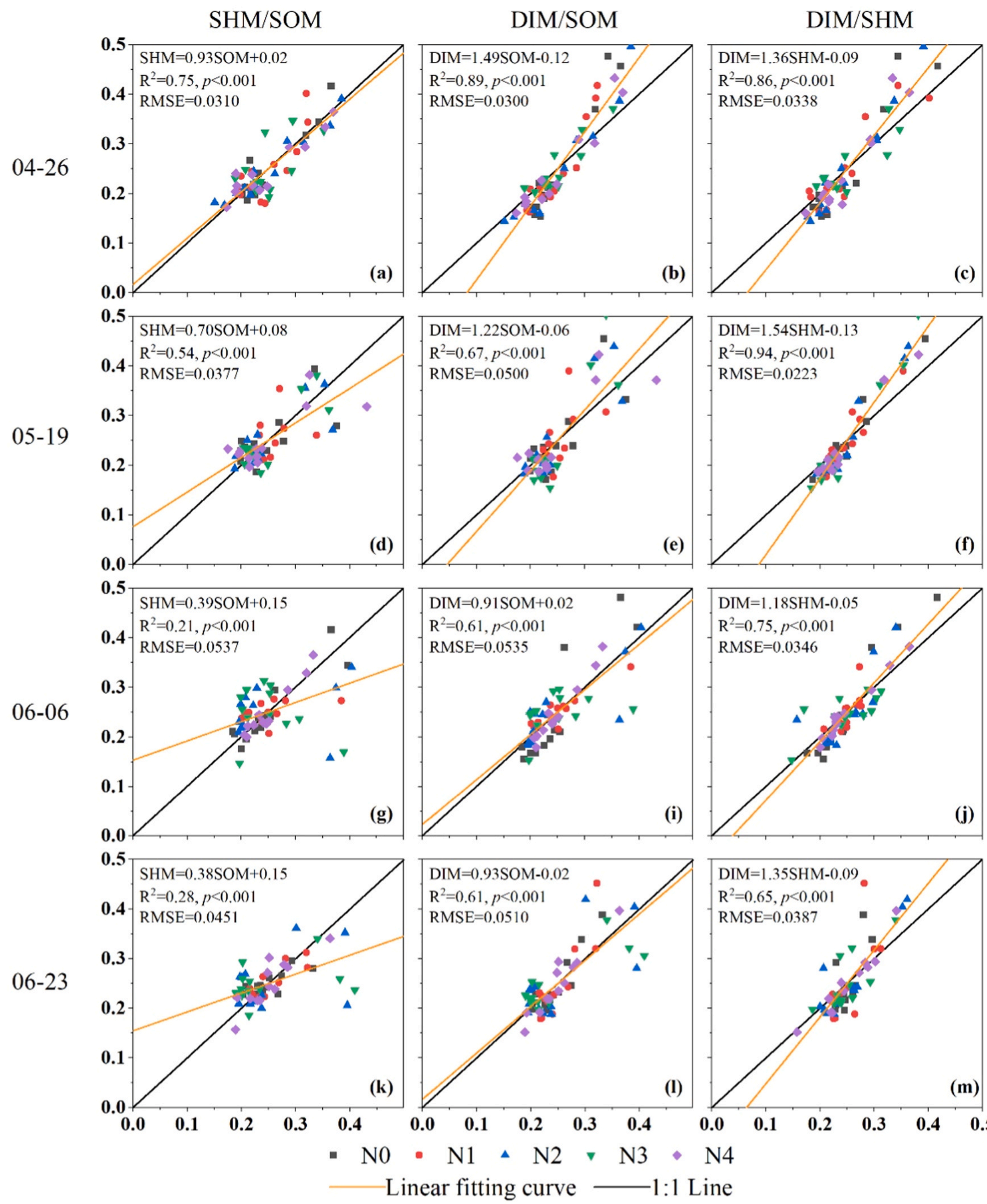

Fig. 5. Linear relationship between the contribution ratios of single $\delta^{18}O$ method (SOM) versus contribution ratios of single $\delta^{2}H$ (SHM) method (SHM/SOM), contribution ratios of single $\delta^{18}O$ method versus double isotope method (DIM/SOM), and contribution ratios of single $\delta^{2}H$ method versus double isotope method (DIM/SHM), respectively. From left to right corresponding to SHM/SOM, DIM/SOM, DIM/SHM. From top to bottom corresponding to different sampling date, 04/26, 05/19, 06/06, 06/23 in 2022, respectively. Black line and yellow line represented the 1:1 fitting line and linear fitting curve, respectively.

3.3. Comparison of MixSIAR model results between three methods

Under different sampling days, the relationships between SHM and SOM were less significant in 05/19 ($R^2 = 0.54^{***}$), 06/06 ($R^2 = 0.21^{***}$), and 06/23 ($R^2 = 0.28^{***}$), respectively (Fig. 5). The regression line of these three days was deviate from the 1:1 fitting line. The average R^2 between DIM/SOM, and DIM/SHM are higher than the R^2 between SHM/SOM. On 04/26, the relationship between DIM/SOM was strongly significant ($R^2 = 0.89$, $p < 0.001$), but the slope of 1.49 deviated from the 1:1 line. As the crop growth stages progress, the RMSE between the DIM and SOM were deviated, and the R^2 decreased in high contribution soil layer. On 06/23, the RMSE between DIM and SOM methods was 0.0510, and $R^2 = 0.61$ ($p < 0.001$). The R^2 of DIM/SHM was greater than that of DIM/SOM on 05/19, 06/06, and 06/23, respectively. Apart from 04/26, the impact of the SOM method on DIM method was greater than the impact of SHM method on DIM method on 05/19 (DIM/SOM slope = 1.22, DIM/SHM slope = 1.54), 06/06 (DIM/SOM slope = 0.91, DIM/SHM slope = 1.18), and 06/23(DIM/SOM slope = 0.93, DIM/SHM slope = 1.35), respectively.

The three methods were in significant agreement at all sampling dates only under N4 treatment (Table 3). There was no significant linear correlation between DIM/SOM under the N3 treatment on 06/06 ($p = 0.25$), neither in DIM/SHM under N0 treatment on 06/23 ($p = 0.52$). The relationship between SHM/SOM was not significant under N1 ($p = 0.21$), N2 ($p = 0.10$) and N3 ($p = 0.11$) treatments, respectively. SHM/SOM also reached lowest R^2 (0.54) and the highest RMSE (0.0537) on 06/06 (Fig. 5).

3.4. Uncertainty of MixSIAR model

3.4.1. Inner group standard deviation

Fig. 6 showed the average standard deviation of three methods under different treatments based on the three repetitions. The four sampling dates showed different magnitudes of standard deviations. On 04/26, the average standard deviations (3.1%) were greater than the other three days among all three methods, and the average standard deviations on 05/19, 06/06 and 06/23 were 2.2%, 2.6%, and 2.6%, respectively. The rank of the standard deviations among all three different methods was DIM > SHM > SOM. Under the N1 treatment, the average standard deviations were the smallest.

Table 3
The correlation coefficient factors of SHM/SOM, DIM/SOM, and DIM/SHM and under different nitrogen treatments (N0 to N4). SHM/SOM stands for the correlation between the contribution ratios of single $\delta^{18}O$ method (SOM) versus contribution ratios of single δ^2H (SHM) method, DIM/SOM stands for the correlation between the contribution ratios of single $\delta^{18}O$ method (SOM) versus double isotope (DIM) method, and DIM/SHM stands for the correlation between the contribution ratios of single δ^2H (SHM) method versus double isotope method (DIM), respectively. The correlation significance shows by "* " when $p < 0.05$, "* *" when $p < 0.01$, and "* ** " when $p < 0.001$. NA means no significance correlation.

	Sampling Date	N0	N1	N2	N3	N4
SHM/ SOM	04/26	0.89***	0.63**	0.93***	0.36*	0.87***
	05/19	0.46**	0.15NA	0.64***	0.70***	0.62**
	06/06	0.84***	0.21NA	0.10NA	0.11NA	0.94***
	06/23	0.58**	0.76***	0.17NA	0.10NA	0.75***
DIM/ SOM	04/26	0.96***	0.86***	0.93***	0.82***	0.93***
	05/19	0.62**	0.35*	0.77***	0.78**	0.72***
	06/06	0.82***	0.85***	0.67***	0.13NA	0.97***
	06/23	0.84***	0.77**	0.52**	0.69***	0.92***
DIM/ SHM	04/26	0.89***	0.81***	0.96***	0.71***	0.90***
	05/19	0.95***	0.88***	0.95***	0.95***	0.96***
	06/06	0.94***	0.48*	0.61**	0.52**	0.98***
	06/23	0.52NA	0.50*	0.81***	0.56**	0.92***

3.4.2. Mean percentage error of different methods

The MPE of different methods was shown on Table 4. In the comparison between single isotope methods, except for the MPE_{SOM} under N4 treatment on 06/06, which had a difference of 0.02 compared to the MPE_{SHM}. The average difference in MPE_{SOM} and MPE_{SHM} on 04/26 was 0.16, which increased to 0.22 on 05/19, and then gradually decreased to 0.11 and 0.08 on 06/06 and 06/23, respectively. The performance of MPE_{DIM} was superior to that of single isotope methods. The sum of MPE_{SOM} ($\delta^{18}O$) - MPE_{DIM} ($\delta^{18}O$) results was 0.19, while the sum of MPE_{SHM} (δ^2H) - MPE_{DIM} (δ^2H) results was 0.37, indicating the DIM method had a better optimization effect on the SHM method. Under different nitrogen treatments, the MPE values did not show a stepwise distribution with the nitrogen gradient. Under the N0 treatment, the performance of different methods was worse than that of the other treatments. The result of the average MPE values on different sampling dates under the SOM method of N0 treatment was significantly large than other treatments. The MPE_{SHM} under N0 treatment were significantly higher than those in the N2, N3, and N4 treatments. The variability of stem and soil water isotopes was also the highest under the N0 treatment (Table 2), indicating different nitrogen application treatments had a significant impact on the model's water distribution accuracy.

4. Discussion

4.1. Wheat root water uptake patters when considering nitrogen treatments

The maximum root depth of crops limits the range of water utilized by crops. Under the condition of limited root length, the proportion of crop water absorption is positively correlated with root length density and dry root weight density (Ma and Song, 2016; Zhao et al., 2018). In dryland agricultural ecosystems, crop root development was influenced by nitrogen fertilization. Previous studies showed that fertilization promoted early root growth at deeper layers (R. Wang et al., 2014; C. Wang et al., 2014), which can effectively enhance drought resistance of the crop by utilizing a larger range of soil water in the early growth stage. Our results showed that surface soil water might influenced the model source tracing results because of the large variations of soil water stable isotope values. There were no significant changes in soil water stable isotopes under different nitrogen treatments, indicating that the variations of soil water isotopic values might be less affected by different nitrogen treatments. The discriminations between wheat xylem water might mainly brought about the different root water uptake distribution results calculated by MixSIAR model. The wheat root water uptake pattern changed under the N0 treatment was relatively smaller (Fig. 4) than that of under N1, N2, N3, and N4 treatment, respectively. Opposed to under N0 treatment, the other treatments revealed a significant concave root water uptake distribution in the transition from shallow layer soil to deep layer soil. This phenomenon possibly due to the limitation of wheat root density under non-nitrogen fertilization conditions. In the later growth period of wheat, such as under N3 and N4 treatments, reducing nitrogen fertilization promoted wheat to use more water from deeper layers of soil compared to that of N1 treatment, which was consistent with another studies on corn under different nitrogen fertilization treatments (Gao et al., 2022). Therefore, different nitrogen fertilization treatments limited the root growth of wheat, thereby limiting their ability to absorb water from deeper soil layers. However, with the growth period elapsing, crops still had the ability to obtain water and nutrients from deeper soil layers to varying degrees. Thus, the deep water extraction and flexible water source selection capabilities might be a reason why N3 treatment showed the largest yield among all nitrogen treatments.

The growth stage was another important factor affecting the root water uptake distribution of wheat. Under the same nitrogen treatment, the water source of wheat crops was mainly from the surface soil in the early stage, and then shifted to use deeper soil moisture in the middle

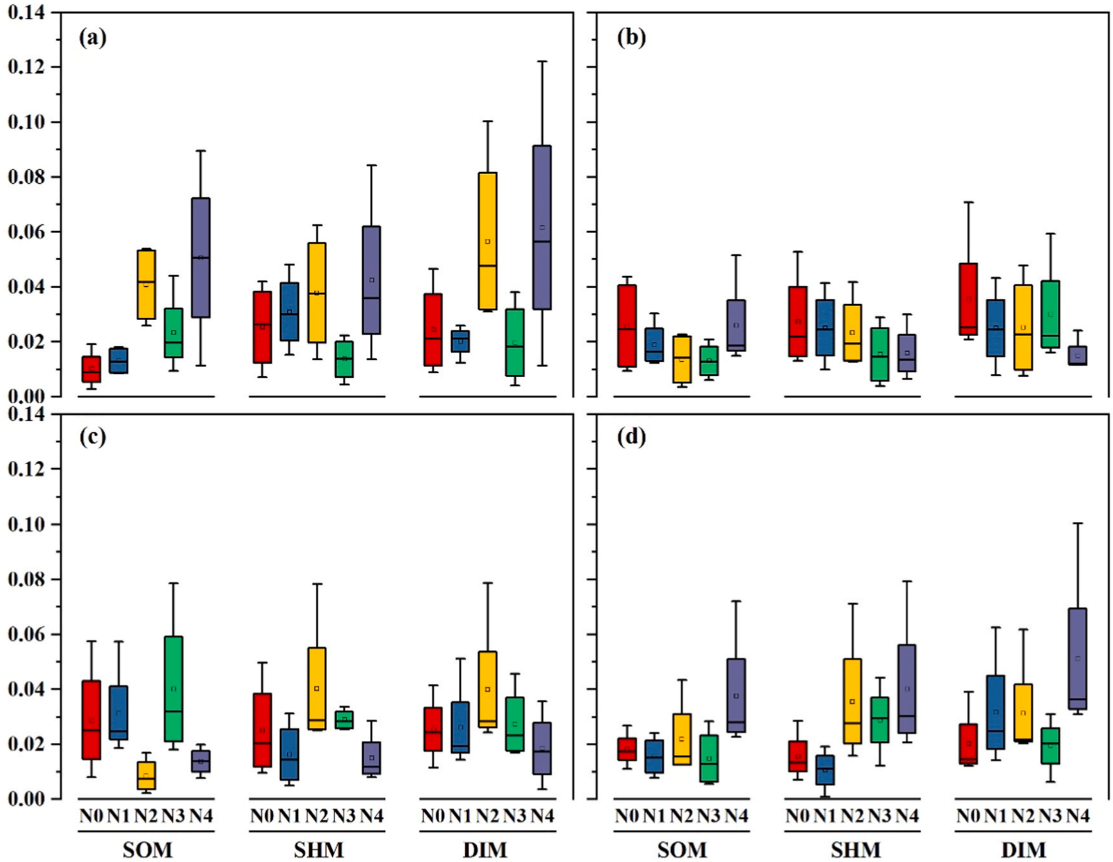

Fig. 6. Differences among root water uptake contributions under different nitrogen treatment (N0-N4), plus under the contribution ratios of single $\delta^{18}O$ method (SOM), the contribution ratios of single $\delta^{2}H$ method (SHM), and the double isotope method (DIM). Each error bar represented the error of each three independent replicate experiments. Figure (a), (b), (c), and (d) are the four sampling dates 04/26, 05/19, 06/06, and 06/23 of 2022 respectively.

Table 4
Performance of different model use approaches, MPE_{DIM}-MPE_{SOM} ($\delta^{18}O$) and SOM_{DIM}-MPE_{SHM} ($\delta^{2}H$) showed the difference of deviation of double isotope method and single isotope method in 2022. And letters in Total showed the significant difference between different nitrogen treatment under the same stable isotope method.

	Method	MPE_{SOM}	MPE_{SHM}	MPE_{DIM}		MPE_{SOM}-MPE_{DIM}	MPE_{SHM}-MPE_{DIM}
		$\delta^{18}O$	$\delta^{2}H$	$\delta^{18}O$	$\delta^{2}H$	$\delta^{18}O$	$\delta^{2}H$
04/26	N0	0.51	0.18	0.47	0.15	0.04	0.02
	N1	0.36	0.12	0.30	0.11	0.06	0.01
	N2	0.21	0.04	0.30	0.11	-0.09	-0.07
	N3	0.06	0.02	0.03	0.02	0.03	0.00
	N4	0.05	0.03	0.04	0.03	0.01	0.00
05/19	N0	0.51	0.12	0.49	0.11	0.02	0.01
	N1	0.23	0.08	0.22	0.06	0.01	0.02
	N2	0.21	0.05	0.19	0.02	0.02	0.03
	N3	0.27	0.09	0.23	0.05	0.04	0.03
	N4	0.28	0.06	0.26	0.04	0.02	0.02
06/06	N0	0.51	0.18	0.43	0.13	0.08	0.05
	N1	0.04	0.03	0.06	0.01	-0.02	0.02
	N2	0.17	0.04	0.19	0.01	-0.02	0.05
	N3	0.07	0.03	0.12	0.05	-0.05	0.03
	N4	0.04	0.06	0.02	0.06	0.01	0.01
06/23	N0	0.32	0.30	0.31	0.28	0.01	0.01
	N1	0.23	0.23	0.20	0.20	0.03	0.03
	N2	0.26	0.03	0.26	0.01	0.00	0.02
	N3	0.08	0.02	0.10	0.00	-0.02	0.02
	N4	0.09	0.03	0.09	0.02	0.00	0.01
Total	N0	0.46a	0.19a	0.42a	0.17a	0.04	0.02
	N1	0.22b	0.11ab	0.20bc	0.10ab	0.02	0.02
	N2	0.21b	0.04b	0.24b	0.03b	-0.02	0.01
	N3	0.12b	0.04b	0.12bc	0.01b	0.00	0.03
	N4	0.11b	0.04b	0.10c	0.03b	0.01	0.01

stage. In the latter stage of the growth period, due to root shrinkage, it turned back to using surface soil water (Zhao et al., 2018). The MixSIAR simulating results of this study once again confirmed the above point of view: the growth period elapsing was another important factor affecting the water source of wheat crops.

4.2. Potential reasons for the biases between different methods of the MixSIAR model

Although the MixSIAR model reduced the uncertainty by inputting prior information (Stock et al., 2018), the root water uptake proportion results were various under SOM, SHM, and DIM methods. The correlation between the results of the two single isotope methods were not significant (Fig. 5), indicating that the water source tracing results of the two isotopes may not be completely consistent. While some studies attributed those differences to the hydrogen isotope fractionation during root water uptake (Li et al., 2007), a more recent study exposed another view of the oxygen isotope fractionation during soil water cryogenic extractions (Chen et al., 2020). Also, some studies explained the differences in water source tracing results between hydrogen and oxygen isotopes from the perspective of cryogenic extraction. They believed some unproper temperature settings during the extraction made it difficult to obtain the bound water, bringing about the biased extraction samples (Gaj et al., 2017; Orlowski et al., 2018). Our study set the extraction time to 3 h, the extraction temperature to 198 °C, which can be considered thoroughly extractions (Jiang et al., 2022). The differences between two methods (SOM and SHM) were various on different growing stage. Two methods were in great consistency on the jointing stage (04/26), but the basis between the two methods was getting greater with the growing stage elapsed (Table 3).

This study considered that the main reason for the basis between the two single isotope methods might be due to the time delay of water transport in wheat crops under different growth conditions. Previous studies showed that the water transport pathway in plants had a time delay (Freyberg et al., 2020). The time difference between absorption and utilization is usually a few minutes, which was usually ignored (Babst et al., 2022). The transpiration rate of wheat crops is a driving factor for the transport of xylem water and is influenced by the growth stage and daily variations (Lu et al., 2023). Although the time of wheat water transport from absorption to the determination point is not as long as trees (Volkmann et al., 2016), the water loss in the transfer process might also cause the problem, in that the stem of wheat mainly consists of a hollow ring of vascular bundles, and water transported through the vascular bundles around the stem (Langensiepen et al., 2014). Previous studies showed that the composition of xylem water isotopes in wheat varies in a day. The longer the retention time of water in the stem of the crop under strong evaporation conditions, the more likely it is to exacerbate the degree of different loss effects of hydrogen and oxygen isotopes, resulting in biases in water source tracing results (Cernusak et al., 2002). Our results also indicated that considering only a single isotope method may not be sufficient to explain the contribution of water sources to plant water, and the DIM method might be a solution to reduce uncertainty.

Different sampling time may also be one of the factors causing differences between the two methods. The error of soil water isotope compositions among three replicated samplings would significantly deliver uncertainties to the MixSIAR model, especially on 04/26 (Fig. 6). In order to obtain more accurate MixSIAR model output results, using synthetic isotopic tracers would be an effective method for increasing the isotopic value differences between water sources (Huo et al., 2020). Previous studies focused more on trees rather than on perennial and annual plants. Compared to perennial plants, wheat is affected by the growing stage. For example, the inner group standard deviation of the three methods was relatively small when the wheat was in the heading stage on 05/19. On the contrary, the double isotope method amplified the inner group standard deviation compared to the single isotope method on the other growth stages. This was more obvious during the jointing stage when the uneven growth of wheat brought about the largest differences in root water uptake distribution results. At the beginning of the wheat growing stage, wheat had different growth and development conditions, leading to greater differences in root water uptake distribution results. At the middle stages of the wheat growth period, the root system stopped growing and became stabilized, leading to lower differences in root water uptake distribution results. At the late stages, however, as the mature root system shriveled and deteriorated (Zhao et al., 2018), the inner group standard deviation increased. We cautiously suggest for subsequent studies to obtain wheat stem samples with similar growth conditions at the beginning of the wheat growing stage.

4.3. Optimal Bayesian mixture model moisture traceability calculation for wheat crop

As our results were shown in Table 4, the DIM method was better than the SOM and the SHM method. Most of the MPE_{DIM} was lower than that of under two single isotope methods. On average, the MPE_{DIM} was 0.04 lower than the MPE_{SOM} under the N0 treatment, and was 0.03 lower than the MPE_{SHM} under the N3 treatment. The MPE_{SHM} was lower than the MPE_{SOM}, indicating the SHM method had higher simulation accuracy than the SOM method. Although recent research suggested that δ^2H may have fractionation during crop soil water uptake (Li et al., 2021), we need to figure out a solution to quantify the δ^2H fractionation during crop soil water uptake in the future.

It was suggested that the double isotope of leaf water showed more informative environmental signals such as atmospheric vapour and relative humidity than the single isotope (Cernusak et al., 2022). The DIM method also provides a better explanation for the possible fractionation in the process of root water uptake and sample condensation vacuum extraction (Chen et al., 2020; Li et al., 2021), so the DIM method would be recommended in root water uptake studies.

4.4. Limitations of this study

Our study provided a new perspective for the introduction of different MixSIAR model parameters into the method, and evaluated the water use strategies of wheat under different nitrogen application conditions. However, some research deficiencies still need to be mentioned. First, there was no evaluation of other crops or other irrigation treatments. Different natural conditions may have different effects on the experimental results (Shen et al., 2015; Wu et al., 2018) under field conditions, so more studies are needed to get a general rule on the effect of nitrogen application on yield. Second, this study did not apply nitrogen application models to justify the nitrogen reduction gradient. In order to obtain the best relationship between yield and nitrogen application, more experimental studies and model simulation results are necessary (Swarbreck et al., 2019). More experimental studies are still needed on the relationship between crop water use pattern and nitrogen treatment results.

5. Conclusion

Based on the field campaign, this study compared the suitability of the single oxygen isotope method (SOM), single hydrogen isotope method (SHM), and double isotope method (DIM) in the Bayesian stable isotope mixing (MixSIAR) model for tracing the origin of water sources, as well as investigated the impact of different nitrogen fertilization treatments. The results showed that the 15% nitrogen reduction treatment exhibited the smallest variation in water source, primarily deriving from the 0–10 cm soil layer from jointing to harvest. In contrast, the 30% nitrogen reduction treatment displayed the greatest variability and deepest soil water uptake pattern. The consistency between the results of the two single-isotope methods under different modeling approaches was higher in the early stages of wheat growth but declined in the later stages. The discrepancy in root water uptake distribution results between the two methods might be influenced by the water transport mechanism in wheat stem and sampling errors. The comprehensive evaluation results indicated that the root water uptake distribution results obtained from hydrogen isotopes were better than those obtained from oxygen isotopes alone. The double isotope method could significantly reduce the uncertainties of the root water uptake distribution results when applying no nitrogen treatment and nitrogen reduction by 30% treatment, although it could potentially amplify errors in the sampling process. To effectively serve the study of water resource utilization in agroecosystems, the growth and development of crops and the errors introduced by the input methods of the model need to be considered when using stable hydrogen and oxygen isotope methods to quantify the root water uptake distribution.

CRediT authorship contribution statement

Du Taisheng: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. **Yingbo Liu:** Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Validation. **Yuan Yusen:** Methodology, Writing – review & editing, Formal analysis, Writing – original draft. **Zhang Liang:** Investigation.

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.

Data availability

Data will be made available on request.

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