CHILEAN JOURNAL
of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Using stable isotopes to study water uptake characteristics of summer corn under different fertilizer treatments

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Received: 19 September 2025; Accepted: 13 January 2026, doi:10.4067/S0718-58392026000200313

ABSTRACT

Water and fertilizer are the major limiting factors in crop production. At present, the utilization of chemical fertilizers is not reasonable, and excessive use of chemical fertilizers makes a serious threat to the environment, while organic fertilizer is a green and sustainable fertilization method for crops. The objective of this study was to determine the water uptake characteristics of corn (*Zea mays* L.) after applying chemical and organic fertilizers, using dual stable isotopes (delta Oxygen-18 [$\delta^{18}O$] and delta deuterium [δD]) to study the main water uptake soil layers and the contributions of soil water at different depths to corn. A field experiment was carried out using a randomized block design in Gaocheng, China. For T1, chemical fertilizers were applied in both wheat (*Triticum aestivum* L.) and corn seasons. For T2, chemical and organic fertilizers were applied during the wheat season, and chemical fertilizers were applied during the corn season. Results showed that water uptake mainly came from the 0-0.2 m depth at the milk growth stage (81%), 0-0.4 m depth at the 6th leaf growth stage (85%), and 0-0.7 m depth at the 9th leaf growth stage (86%), 13th leaf growth stage (91%), tassel growth stage (89%), silking growth stage (87%), blister growth stage (95%), and dough growth stage (94%). Fertilization led to clear differences in the proportional contribution of soil water from 0-0.2 m (average 35% and 44% for chemical and organic fertilizer, respectively), 0.2-0.4 m (25% and 21%), and 0.7-1.2 m (10% and 5%). The manure fertilizer reduced soil bulk density, and increased soil stable aggregates, saturated water content, soil porosity, and water retention capacity of shallow soil. Our results provide a theoretical basis for developing a reasonable fertilization plan combining inorganic and organic fertilizers in arid areas.

Key words: Corn, fertilization mode, hydrogen and oxygen stable isotopes, water uptake.

INTRODUCTION

Organic fertilizer, as a green, high-yield, and sustainable soil-improvement tool, has been widely used in crop production (Zhang et al., 2012). The combined application of organic and inorganic fertilizers can optimize the physical and chemical properties of soil, such as improve the types and quantity of microorganisms in the soil; promote the decomposition of organic matter and transformation of nutrients in the soil (Li et al., 2023), and enhance the fertilizer supply in the soil (Liang et al., 2019); balance inorganic nutrients such as N, P, and K in the soil; achieve a balance between organic and inorganic components (Xiang et al., 2022; Zhang et al., 2023), which is advantageous to crop growth (Wang et al.,2012; Maharjan et al., 2021; Mu et al.,2023; Delgado et al., 2024). Organic fertilizer reduces soil bulk density in the plough layer, improves the soil aggregate structure, and increases soil porosity (Yang et al., 2018). This porous soil structure has an important impact on improving soil water storage capacity and increasing precipitation infiltration, which helps to retain water in the crop zone, reduce soil water evaporation, coordinating the contradiction between crop water demand and soil water supply (Wang et al., 2012; Zheng et al., 2017), and laying the foundation for high yield and water use efficiency (WUE) (Zhai et al., 2022). Organic matter can indeed alter soil structure, but does this change contribute to the uptake and utilization of water by corn? Given the limited water resources in northern China, studying the characteristics of corn root-water uptake after applying organic fertilizers can help develop reasonable fertilization and irrigation plans, and provide theoretical basis for sustainable food production in similar region.

Stable isotopes (δD and $\delta^{18}O$) have been widely used to study contaminant sources (Zaryab et al., 2024), hydrologic cycles (Gat, 1996), and water uptake by crops (Ma and Song, 2016; Wu et al., 2016). Tracing stable isotopes is an unintrusive and effective way to assess water uptake patterns (Ehleringer and Dawson, 1992). Zimmermann et al. (1967) reported that no fractionation of isotopes occurs during water transfer from the soil to the plant. Therefore, the isotopic composition of water in crops can be used to infer the depth of water uptake (Zhang et al., 2024). The MixSIAR Bayesian isotope mixing model allows for the input of multiple isotopes using raw source data while addressing hierarchical random or fixed effects in the analysis (Stock and Semmens, 2013). This model account for uncertainties in isotope values and the estimates of water source contributions (Erhardt and Bedrick, 2013), and have been extensively used to quantitatively identify water source used by plant (Yang et al., 2015). Researcher found that most of C_4 crop absorb more water from shallower depth of soil than the C_3 crop, and plants mainly absorb deeper soil water in dry season but shallower soil water in wet season (Dawson and Pate, 1996). Ma and Song (2016) used stable isotopes to determine seasonal variation in water uptake in corn under different N fertilization treatments, and the contributions of soil water at different depths to water uptake were quantified using MixSIAR. Zhao et al. (2018) used the stable isotopes $\delta^{18}O$ and $\delta^{2}H$ to investigate the water uptake patterns of a corn and winter wheat rotation system in the North China Plain. Asbjornsen et al. (2007) used stable isotopes to determine corn mainly obtains 45% of its water from the 0-0.2 m soil layer. However, the water uptake rate of crops varies with the growth stage. Wang et al. (2010) indicated that the shifting water uptake layer lies in the silking and black layer growth stages for corn. They found that corn used 96%-99% of water from the 0-0.2 m soil layer at the jointing stage, 58%-85% of water from the 0.2-0.5 m soil layer at the silking stage, and 69%-76% of water from the 0-0.2 m soil layer at the black layer growth stage (Wang et al., 2010).

Organic fertilizer changes the distribution characteristics of soil moisture, the combination of organic and inorganic fertilizers can not only increase crop yield, but also improve the proportion of soil water supply and soil water use efficiency, playing the role of “adjusting water with fertilizer” (Wang et al., 2012; Zhang et al., 2016; Bottinelli et al., 2017; Zhai et al., 2022). Previous studies have mostly applied stable isotope techniques to investigate the soil water uptake characteristics under different N fertilizer application rates (Ma and Song, 2016) and different types of crops (Wang et al., 2010; Zhao et al., 2018); however, there are few studies on the physiological characteristics of corn root-water uptake and the seasonal changes under the condition of applying organic fertilizer, especially the main soil layers of corn water uptake and the contribution of water uptake from different soil depths as the growth stage changes.

In this work, we used the stable isotopes δD and $\delta^{18}O$ and MixSIAR to quantify the water uptake patterns of corn under two different treatments (organic fertilizer and inorganic fertilizer) during 2020 in Gaocheng, China. We quantified the contribution of water uptake from different soil depths, evaluated the relationship between water uptake and soil moisture, and compared the differences in uptake between treatments. This study will provide a theoretical basis for developing a reasonable fertilization plan combining inorganic and organic fertilizers in arid areas.

MATERIALS AND METHODS

Field experiments with corn (*Zea mays* L.) were conducted at the Gaocheng Experimental Base of the Institute of Cereal and Oil Crops, Hebei Academy of Agricultural and Forestry Sciences, during 2020. The experimental site is located at the base of Mount Taihang (38°41′ N, 116°85′ E) on high-yield farmland on the North China Plain. The staple crops in this region are wheat (*Triticum aestivum* L.) and corn (grown in rotation), harvested twice per year. The area is in a monsoon climatic zone with a mean annual precipitation of about 484 mm, and the average annual frost-free period is 144 d. The well-drained silt soil with a deep profile here is very suitable for crop growth. Average field capacity is 22.2% for the 1.2 m profile (Table 1). The organic matter content of the soil is 15.5 g kg^{-1} in the 0-0.2 m tillage layer. The total N content is 1.0 g kg^{-1} and the available P and K contents are 22.0 and 81.0 mg kg^{-1}, respectively.

Table 1. Field capacity of soil at Gaocheng station.

Soil depth, m	0-0.2	0.3-0.4	0.5-0.6	0.7-0.8	0.9-1.0	1.1-1.2
Field capacity, %	22.3	18.2	22.4	21.7	22.8	25.5

Experimental design

A field experiment with two treatments (T1 and T2) was carried out using a randomized block design. For T1, wheat season and corn season were grown with chemical fertilizer only from 2017 to 2020. For T2, wheat season was grown with chemical and organic fertilizers, whereas corn season was grown with chemical fertilizer from 2017 to 2020. After running fertilization treatments on wheat and corn for 3 yr, the effect of previous application of organic fertilizer on corn water uptake was studied during 2020 growing season. Chemical fertilizer (N-P_2O_5-K_2O: 20%-26%-8%) and organic fertilizer were used as base fertilizer. The organic fertilizer used in this study was produced from cow manure and contained 45% organic matter, 2.48% N, 0.27% P_2O_5, and 0.96% K_2O. The basal organic and chemical fertilizers were incorporated into the 0-0.2 m soil layer using a rotary tiller just before wheat sowing. Details on the amount and type of fertilizer applied in each treatment are given in Table 2. The planting density of corn was 67 500 plants ha^{-1} with a row spacing of 0.6 m. Each treatment had three replicates with a corresponding plot area of 4.2 × 15 m = 63 m^2. During the wheat growth period, 225 kg ha^{-1} urea (46% N) was added at the jointing growth stage. After harvest, corn was sown without tillage, and 225 kg ha^{-1} urea (46% N) was applied before irrigation or rainfall in the 12^{th} leaf stage. In 2020, the corn crop was not irrigated, and precipitation during the growth period was 272.9 mm (Figure 1); this was a dry year. The corn was sown on 18 June and harvested on 30 September.

Table 2. Types and contents of base fertilizer of different treatments. CF: Chemical fertilizer.

Treatments	Winter wheat	Corn
T1	CF 600 kg ha^{-1}	CF 375 kg ha^{-1}
T2	CF 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung	CF 375 kg ha^{-1}

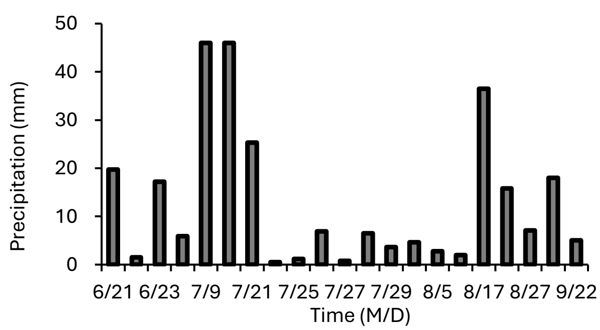

Figure 1. Precipitation during corn growing period in 2020.

Water contents

Rainwater. A rain collector consisting of a polyethylene bottle and funnel was placed outside, and a ping-pong ball was positioned at the funnel mouth to prevent evaporation during rainfall. After each rainfall event, rainwater was collected and immediately transferred to a bottle, sealed, and stored.

Stem water. Stem water was sampled at different growth stages, as summarized in Table 3. To avoid isotope fractionation caused by plant transpiration, stems containing no chlorophyll at 0.05 m above the soil surface were collected, placed in 5 mL airtight vials sealed with parafilm, and frozen (-15 to-20 °C) before isotopic analysis. Stem water was extracted using an automatic cryogenic vacuum distillation system (LI-2000, LICA United, Beijing, China). The process took 3 h and had a > 98% extraction rate, which is sufficient for obtaining unfractionated water samples.

Table 3. Growing stage and sampling date of corn in 2020. V6: Sixth leaf; V9: ninth leaf; V13: thirteenth leaf; VT: tassel; R1: silking; R2: blister; R3: milk; R4: dough.

Growing stage	V6	V9	V13	VT	R1	R2	R3	R4
Date	9 Jul	19 Jul	30 Jul	5 Aug	15 Aug	26 Aug	6 Sep	16 Sep

Soil water. Soil water was sampled (on the same dates on which stems were sampled) at depths of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 m using a soil auger. The samples were stored, processed, and analyzed in the same way as the stem samples. Three locations at 4 m intervals along a seeding row were chosen for sampling.

The level of soil water in each plot was measured using the gravimetric method. Sampling times and points were the same as those for isotope sampling. Volumetric soil water content (θv) was calculated as θv = θw × γ, where θw is gravimetric water content (w/w, %) and γ is the soil bulk density (g cm^{-3}).

δD and $\delta^{18}O$ analyses

The δD and $\delta^{18}O$ levels were measured from all water samples via wavelength scan-cavity ring down spectrometry using a liquid water isotope analyzer (L-2120i, Picarro, Santa Clara, California, USA) and using the International Atomic Energy Agency (IAEA; Viena, Austria) protocol for isotopic analysis of liquid water (Goebel et al., 2015). The results were expressed as δ-values, calculated as follows: δ (‰) = (Rsample – Rstandard)/Rstandard × 1000, where R is the $^{18}O/^{16}O$ or D/H ratio. The measurement precision was < 0.5‰ for δD and < 0.2‰ for $\delta^{18}O$.

The direct inference method was used to determine the main water source for corn growth. This method is based on the assumption that no isotope fractionation occurs during root water uptake; thus, stem water can be considered a mixture of water sources (Zhao et al., 2018). Therefore, by comparing the isotope composition of stem water and soil water from different depths, the main water sources used during each stage of growth can be implied.

Soil physical properties

To measure the particle size distribution and soil aggregate stability, samples were taken from the topsoil layer with a shovel and air-dried at room temperature. To study bulk density, saturated conductivity, and soil water characteristics, undisturbed soil was taken using cutting rings. To assess soil porosity, undisturbed soil cores were collected using polyvinyl chloride tubes (diameter 0.11 m, height 0.2 m). The tubes were gently pushed into the topsoil and excavated with a spade without compaction. Soil cores were wrapped with plastic film and stored at 4 °C. Care was taken to not disturb the soil structure during sampling and transportation.

The cutting ring method was used to determine soil bulk density (Pan et al., 2019). To measure soil porosity, an undisturbed soil core was scanned with a medical computed tomography device at Shijiazhuang Third Hospital. The images were processed and visualized using the open-source software ImageJ ver.1.44 and the ImageJ 3D viewer (National Institutes of Health [NIH], Bethesda, Maryland, USA) (Schneider et al., 2012). Soil particle size distribution was determined using a laser particle sizer (Mastersizer 3000, Marlven Panalytical, Malvern, Worcestershire, UK). Soil aggregates were extracted by wet-sieving soil samples through 2, 1, 0.25, and 0.053 mm sieves. Aggregate extraction was performed using a soil aggregate analyzer (TTF-100, Shangyushunlong, Shaoxing, Zhejiang, China). The centrifugal method was used to obtain a soil water characteristic curve, from which saturated water content and available water capacity were determined. The falling head permeable test and constant head method were used to measure saturated conductivity.

Soil nutrients

Total N, available P, available K, available N, and organic matter were measured using the Kjeldahl apparatus, the Olsen method, flame photometry, alkaline hydrolysis diffusion, and the $K_2Cr_2O_4$ volumetric method, respectively (Pan et al., 2019).

Proportional contribution of water sources

The proportional contribution of each water source to the corn crop was quantified using a Bayesian mixing model (MixSIAR 3.1.7; Scripps Institution of Oceanography, University of California, San Diego, California, USA) (Stock et al., 2018) based on the mass balance of isotopes. Measured dual isotope values (δD and $\delta^{18}O$) of stem water and soil water at different depths were loaded into MixSIAR as original raw data. The discrimination values were set to zero for both δD and $\delta^{18}O$, because of the assumption that there is no isotope fractionation during water uptake. Individual effects as a random occurrence were included in all analyses. The following settings were employed: MCMC run length, "very long"; error structure options, "residual error" and "process error"; and prior, "uninformative/generalist." The Gelman-Rubin and Geweke diagnostic tests were used to determine whether the model converged. The model solutions are presented as medians with standard deviations (SDs).

Yield determination

At physiological maturity, all plants in a 1.8 × 10 m area containing the middle rows of each plot were harvested, and the grain yield was determined under the condition of 14% grain water content.

Statistical analysis

Data were subjected to ANOVA using SPSS ver. 19.0 (SPSS, Chicago, Illinois, USA). Graphs were plotted in Excel 2007 (Microsoft, Redmond, Washington, USA). The proportional contributions of water sources were quantified using a Bayesian mixing model (MixSIAR 3.1.7) based on the mass balance of isotopes. Means of treatments were compared using post hoc Tukey's honestly significant difference (HSD) tests at $P < 0.05$.

RESULTS

Isotopic composition of water

Isotope $\delta^{18}O$ in precipitation ranged from-11.5‰ to-3.4‰, with a mean value of-7.0‰ (SD 2.5‰), whereas δD ranged from-85.0‰ to-21.3‰, with a mean value of-49.6‰ (SD 19.0‰) (Table 4). The local meteoric water line (LMWL) was obtained from local precipitation samples and could be expressed as $\delta D = 7.43 \times \delta^{18}O + 2.05$ ($R^2 = 0.96$; Figure 2). The slope value 7.43 was lower than the value of 7.94 derived from the global meteoric water line (GMWL) expressed as $\delta D = 7.94 \times \delta^{18}O + 3.92$ (Liu et al., 2014), based on data collected by the Beijing station of the Chinese Network of Isotopes in Precipitation. The smaller slope of the LMWL was ascribed to the fast evaporation of falling raindrops in this study.

The range of $\delta^{18}O$ in soil water was-11.0‰ to-4.1‰, with a mean value of-8.1‰ and an SD of 1.3‰, whereas δD ranged from-81.7‰ to-36.4‰, with a mean value of-64.2‰ and an SD of 8.9‰ in T1 (Table 4). In soil water, $\delta^{18}O$ ranged from-11.5‰ to-2.4‰, with a mean value of-8.5‰ and an SD of 1.2‰, whereas δD ranged from-92.5‰ to-42.0‰, with a mean value of-67.6‰ and an SD of 8.2‰ in T2. Stable isotopes in soil water had a scattered distribution, and the slope of the soil water δD-$\delta^{18}O$ relationship (6.34) for T2 was lower than that for the soil water line (SWL) for T1 (6.54; Figure 2), indicating a strong evaporation effect on soil water. Comparing the results to isotopes in precipitation, we determined that the soil water mainly came from replenishment by atmospheric precipitation. This result complements the fact that no irrigation was applied.

Isotope levels were high in topsoil and decreased with soil depth (Figure 2). The δD and $\delta^{18}O$ values for soil water in the 0.8-1.2 m layer were uniform with average values of 9.3‰ for $\delta^{18}O$ and 72.3‰ for δD for T2, and 9.1‰ for $\delta^{18}O$ and 72.0‰ for δD for T1. Levels were higher for T1 than for T2, particularly in the upper 0-0.5 m layer (Table 4). The slope for the SWL was lower than that for the LMWL, due to stronger soil evaporation. Furthermore, the evaporation lines (Table 5) for soil water at depths of 0-0.05 m and 0.05-0.1 m indicate that the slope was significantly larger for T2 than for T1. This also indicates that a combination of straw return and manure application results in better performance in terms of decreasing soil water evaporation.

Mean δ18O and δD values for stem water were, respectively,-7.4‰ and-59.4‰ for T2 and-8.3‰ and-66.5‰ for T1. The values fell near the fitting line of the δD-$\delta^{18}O$ relationship (Figure 2). The isotope values for stem water were similar to those for soil water in the 0-0.7 m layer for T1 and 0-0.5 m layer for T2, indicating that the principal source of stem water was soil water in the upper soil layer.

Table 4. General characteristics of isotopic compositions in water samples across all sampling times. T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Treatments	Moisture source	Soil depth (m)	$\delta^{18}O$ (‰) Max.	Min.	Mean	SD	δD (‰) Max.	Min.	Mean	SD
	Precipitation		-3.36	-11.46	-6.95	2.50	-21.32	-84.96	−49.58	18.95
T1	Soil water	0.05	-4.53	-8.25	-6.66	1.20	-49.07	-66.54	−57.92	6.71
		0.1	-5.78	-9.81	-7.60	1.27	-49.76	-75.57	−61.84	8.54
		0.2	-4.05	-10.94	-7.19	1.79	-36.37	-81.67	−58.15	11.89
		0.3	-5.04	-9.21	-6.98	1.32	-44.42	-70.87	−56.20	8.84
		0.4	-5.29	-8.12	-6.60	0.90	-43.42	-62.03	−52.73	6.30
		0.5	-6.22	-8.04	-7.21	0.53	-51.23	-63.15	−57.45	3.73
		0.6	-6.79	-8.91	-8.07	0.53	-58.59	-69.48	−63.86	3.28
		0.7	-8.09	-9.00	-8.59	0.28	-63.67	-70.65	−67.44	2.07
		0.8	-8.23	-9.41	-8.90	0.33	-67.78	-72.12	−69.79	1.11
		0.9	-8.49	-9.54	-9.07	0.35	-68.11	-72.87	−70.54	1.55
		1.0	-8.44	-10.00	-9.16	0.44	-68.55	-76.65	−72.05	2.53
		1.1	-8.42	-9.72	-9.16	0.45	-68.77	-76.13	−72.14	2.22
		1.2	-8.49	-9.82	-9.28	0.44	-68.99	-75.32	−72.98	1.92
		Total	-4.05	-10.94	-8.05	1.32	-36.37	-81.67	−64.16	8.86
	Stem water		-6.43	-8.86	-8.31	1.56	-52.86	-63.36	−66.47	12.47
T2	Soil water	0.05	-2.38	-11.48	-7.19	2.42	-41.97	-92.48	−61.42	15.08
		0.1	-6.49	-11.07	-8.46	1.52	-53.25	-88.07	−67.08	11.74
		0.2	-4.12	-10.48	-8.35	1.88	-44.04	-80.21	−67.14	11.04
		0.3	-7.41	-9.21	-8.55	0.55	-61.43	-73.62	−67.23	3.27
		0.4	-5.58	-9.26	-7.39	0.99	-48.92	-71.03	−59.00	6.12
		0.5	-7.04	-8.73	-7.80	0.58	-55.54	-68.73	−61.54	4.24
		0.6	-7.62	-9.61	-8.27	0.50	-61.12	-75.94	−65.12	4.10
		0.7	-8.05	-10.00	-8.80	0.61	-63.13	-80.48	−68.86	5.32
		0.8	-8.58	-10.37	-9.17	0.46	-68.74	-80.95	−71.46	3.10
		0.9	-8.49	-10.05	-9.21	0.34	-66.01	-80.27	−72.16	3.53
		1.0	-8.64	-9.96	-9.27	0.35	-67.63	-79.96	−72.66	3.21
		1.1	-8.83	-9.84	-9.34	0.29	-69.46	-79.33	−73.03	2.95
		1.2	-8.41	-9.72	-9.25	0.37	-68.51	-78.63	−72.37	3.10
		Total	-2.38	-11.48	-8.54	1.23	-41.97	-92.48	−67.62	8.18
	Stem water		-4.80	-9.28	-7.42	1.12	-45.55	-77.37	−59.40	7.78

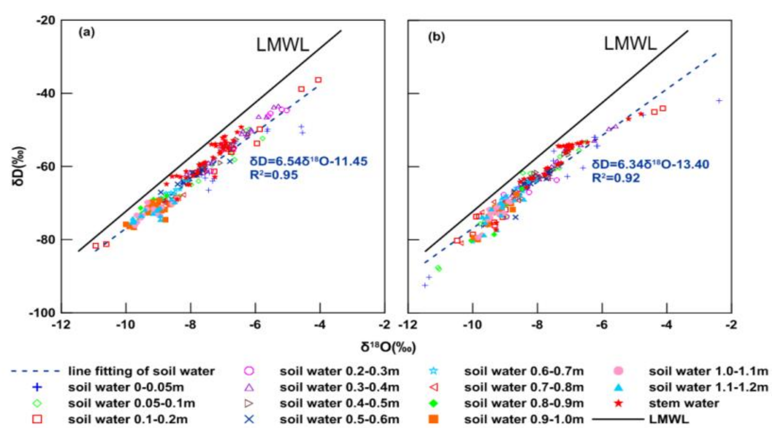

Figure 2. Dual stable isotopes (δD-$\delta^{18}O$) relationship based on water samples from T1 (a) and T2 plots(b). LMWL: Local meteoric water line. T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Table 5. Evaporation line of soil water. T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Treatments	0-0.05 m	0.05-0.1 m
T1	$\delta D = 5.0304\ \delta^{18}O - 24.418$ $R^2 = 0.8114$	$\delta D = 6.5506\ \delta^{18}O - 12.063$ $R^2 = 0.953$
T2	$\delta D = 5.961\ \delta^{18}O - 18.541$ $R^2 = 0.914$	$\delta D = 7.5748\ \delta^{18}O - 2.9997$ $R^2 = 0.9628$

Variation in the depth of water uptake

Figure 3 shows the trend lines of δD and $\delta^{18}O$ with soil water depth (where the vertical dotted line is the isotopic composition of stem water sampled on the same date as soil water). The trends for both isotopes are roughly the same, and the majority of intersections between the stem and soil water compositions appear at the same depths.

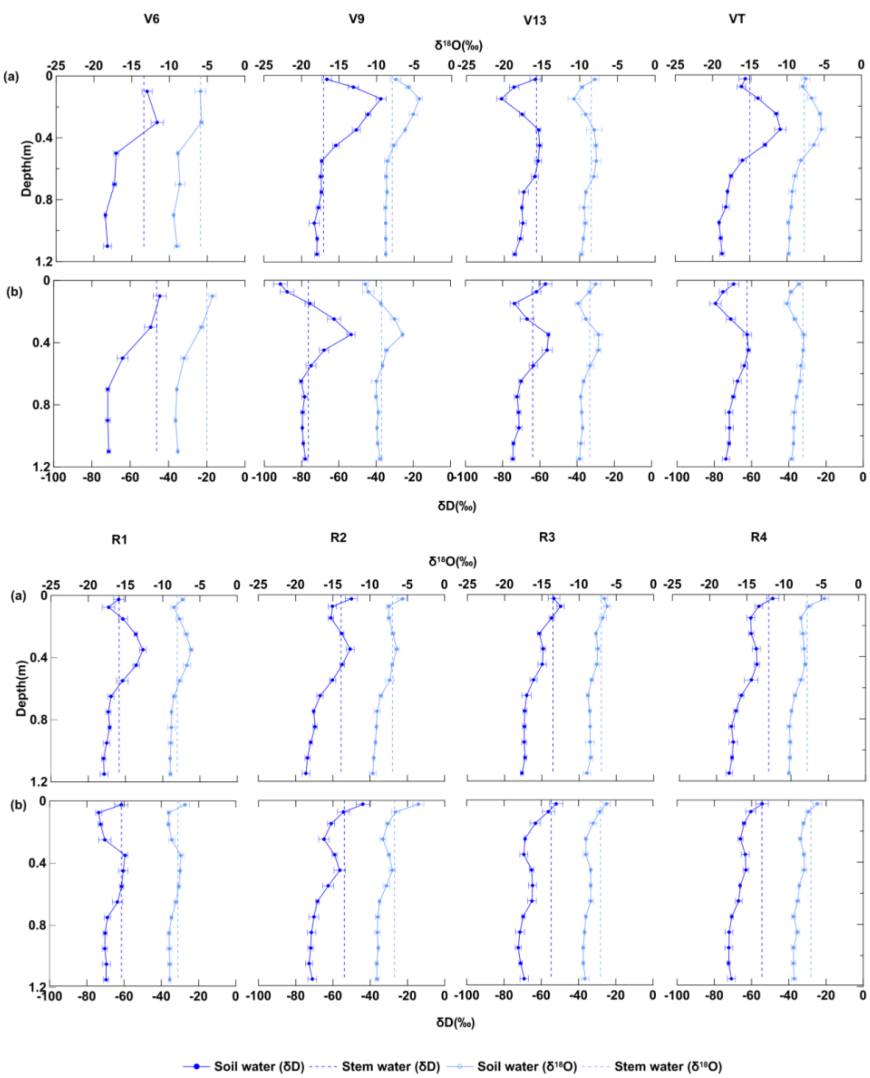

Figure 3. Dual stable isotopes (δD and δ18O) in stem water and soil water at different growth stages under T1 (a) and T2 (b). V6: Sixth leaf; V9: ninth leaf; V13: thirteenth leaf; VT: tassel; R1: silking; R2: blister; R3: milk; R4: dough; T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Take $\delta^{18}O$ as an example. The maximal water uptake depth was observed at 0-0.3 m at the V6 growth stage for both treatments. The predominant water uptake depths were found at 0-0.1 and 0.6-1.2 m for T1 and 0-0.2 and 0.5-1.2 m for T2 at the V9 growth stage; the respective depths were 0.3-0.8 m for T1 and 0.3-0.7 m for T2 at the V13 growth stage, 0-0.2 and 0.5-0.7 m for T1 and 0.3-0.8 m for T2 at the VT growth stage, 0-0.2 and 0.6-1.2 m for T1 and 0.3-0.7 m for T2 at the R1 growth stage, and 0.1-0.6 m for both treatments at the R2 growth stage. At the R3 growth stage and mature stage R4 growth stage, there was a notable difference in the uptake depth, 0-0.2 m for T2 and 0-0.6 m for T1. Overall, the water uptake depth was deeper under T1 than under T2. This indicates that the application of organic fertilizers reduces water consumption in deep soil layers.

Proportional contributions of soil water at different depths to corn

The proportional contributions of soil water were quantified using the MixSIAR model. The contribution from the 0-0.7 m depth was large (Figure 4); below that depth, there were nonsignificant differences based on isotope composition. Thus, the 0.7-1.2 m soil layer was combined for analysis, and the values of δD and $\delta^{18}O$ were averaged.

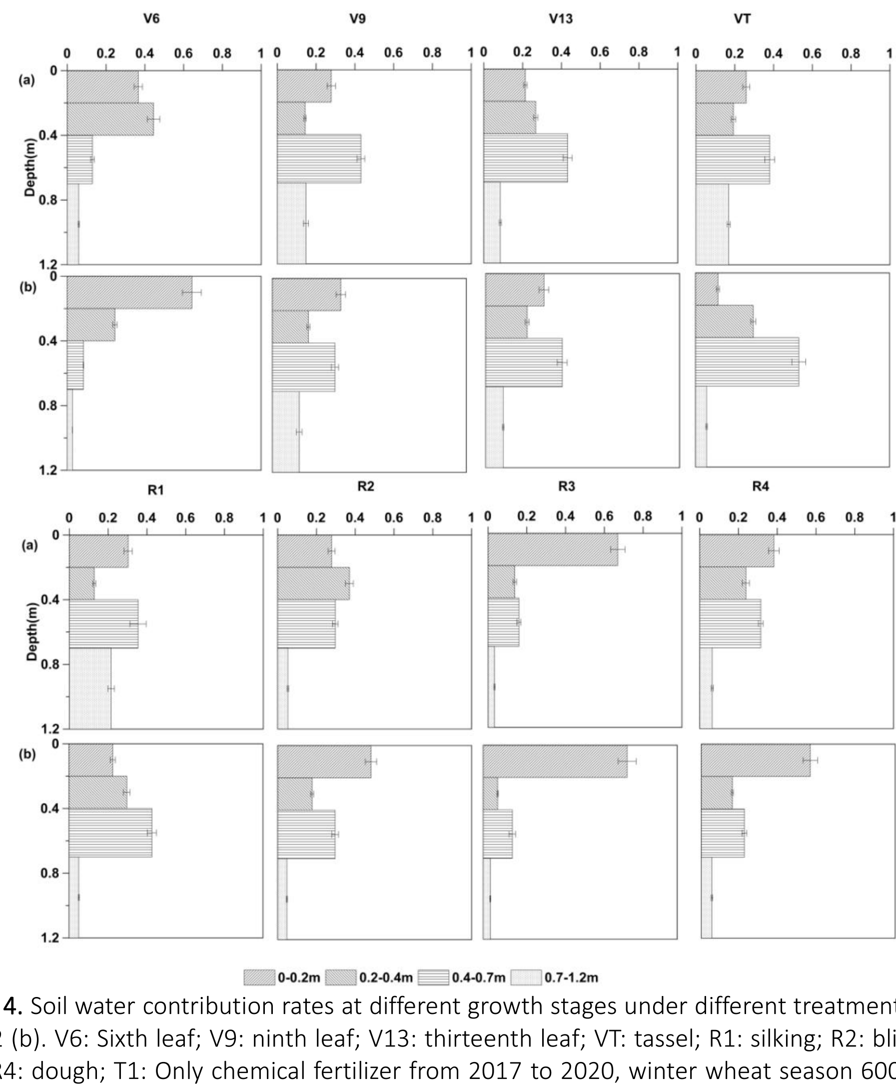

Figure 4. Soil water contribution rates at different growth stages under different treatments: T1 (a) and T2 (b). V6: Sixth leaf; V9: ninth leaf; V13: thirteenth leaf; VT: tassel; R1: silking; R2: blister; R3: milk; R4: dough; T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

The average contribution of soil water from the 0-0.2, 0.2-0.4, 0.4-0.7, and 0.7-1.2 m layers was 40.0%, 23%, 30%, and 7%, respectively. The contribution from 0-0.7 m accounted for 93% of the total. Corn mainly used soil water from depths of 0 to 0.2 m at the V6 (51%), V9 (35%), R2 (38%), R3 (71%), and R4 (47%), and from 0.4 to 0.7 m at the V13 (41%), VT (46%), and R1 (39%). The contribution from 0.2-0.4 m decreased from the V6 to the V9, remained relatively constant from the V13 to the R2, and then decreased thereafter. The maximal contribution from 0.2-0.4 m appeared at the V6 (35%). The contribution from 0.7-1.2 m increased from the V6 to the R1, and then decreased through the remaining stages. A higher contribution (average 12%) from 0.7-1.2 m appeared from the V9 to the R1.

The water uptake patterns were notably different between the two treatments, particularly for the 0-0.2 and 0.7-1.2 m layers (Figure 4). The mean contribution of soil water from the 0-0.2 m layer was 35% and 44% for T1 and T2 respectively, and T2 was 1.3 fold that of T1, with the difference being greatest at the V6 and R2 (1.8 and 1.7 fold differences, respectively). The mean contribution from the 0.7-1.2 m layer was 10% and 5% respectively, and T1 was 2.0 fold that of T2, with the greatest differences observed at the R1, VT, and V6 (4.3, 3.0, and 2.2 fold differences, respectively). These data indicate that water uptake at different stages was notably different between treatments, particularly from the 0-0.2 and 0.7-1.2 m soil layers. These results further confirm the inference that manure application significantly reduces soil water use at deeper soil layers and improves water use from shallow soil layers.

The changes in soil water contribution rate were roughly the same for the 0.2-0.4 m and 0.4-0.7 m layers. The contribution from the 0.2-0.4 m layer under T2 were 1.5 and 2.3 fold that under T1 at the VT and R1, respectively (1.4 and 1.2 fold that under T1 for 0.4-0.7 m). The rate was significantly lower under T2 than under T1 for the other stages, or similar, depending on the stage.

The changes in soil water distribution were consistent with the seasonal variation in water uptake patterns estimated by the MixSIAR model. Specifically, the soil water contribution rates changed obviously along with soil moisture during some critical growth stages. The moisture level in the 0-0.2 m soil layer was mostly higher under T1 than under T2, whereas that in the 0.7-1.2 m layer was mostly lower under T1 (Figure 5). These results confirm that, under T1, the corn absorbed more water from the deep layer than from the surface layer.

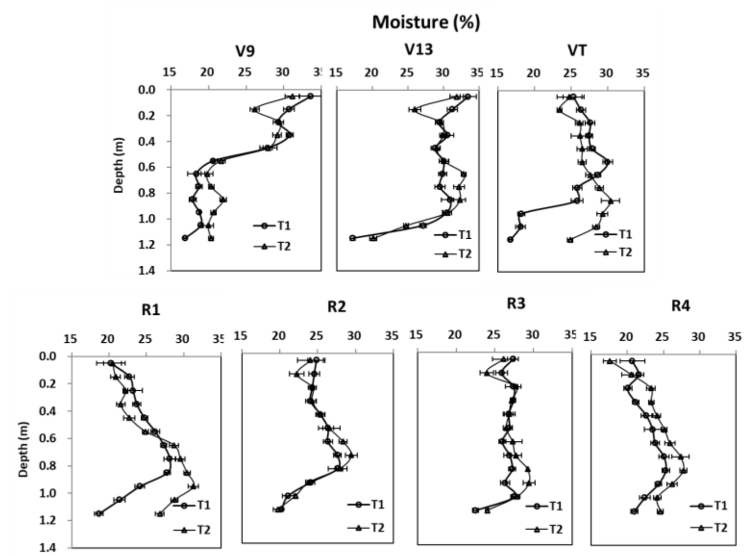

Figure 5. Variation in vertical distribution of soil moisture at different growth stages. T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Soil physical properties and grain yields

The results showed that organic fertilizer led to a higher proportion of aggregates with a particle size of 0.02-2 mm (60%) and 0.002-0.02 mm (38%), significantly higher than that aggregates with a particle size of < 0.002 mm (3%) (Table 6). The T2 significantly increased the content of aggregates with a particle size of < 0.002 mm, but not those of aggregates with other particle sizes. Organic fertilizer also led to significantly more stable aggregates in soil water, lower soil bulk density, significantly higher saturated water content and saturated conductivity, and higher soil porosity in the 0-0.2 m layer (Figure 6). The high porosity indicates that the soil had strong water-holding capacity.

Table 6. Physical properties at 0-0.2 m soil layer of different treatments. Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$). Ks: Saturated conductivity; PAWC: plant available water capacity; T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

Treatments	Particle size distribution of mechanical stable aggregates (%) < 0.002	0.002-0.02	0.02-2	> 0.25 mm water stable aggregates	Bulk density	Saturated water content	25 °C Ks	PAWC	Yields
	mm	mm	mm	%	g cm^{-3}	%	cm h^{-1}	g cm^{-}	kg ha^{-1}
T1	2.2^{b}	38.0^{a}	59.8^{a}	9.15^{b}	1.4^{a}	40.84^{b}	0.09^{b}	0.10^{b}	8089.5^{b}
T2	3.0^{a}	37.6^{a}	59.4^{a}	20.27^{a}	1.3^{b}	46.35^{a}	5.12^{a}	0.12^{a}	8467.3^{a}

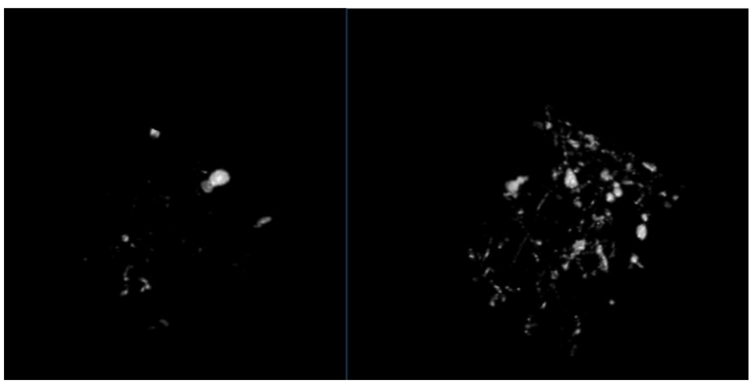

Figure 6. Three-dimensional porosity structure of soil under different fertilization treatments: T1 (left) and T2 (right). T1: Only chemical fertilizer from 2017 to 2020, winter wheat season 600 kg ha^{-1}, corn season 375 kg ha^{-1}; T2: chemical and organic fertilizers from 2017 to 2020, winter wheat season 600 kg ha^{-1} + 4500 kg ha^{-1} dried cow dung, corn season 375 kg ha^{-1}.

DISCUSSION

Spatial distribution of soil moisture and dynamic shifts in maize water uptake layers

In the current study, major changes in soil moisture during the 2020 season occurred in the 0-0.7 m layer, especially in the 0-0.2 m layer. Regarding each developmental stage, the water taken up mostly came from the 0-0.2 and 0.2-0.4 m layers at the 6th leaf growth stage, the 0.4-0.7 m layer at the 9th leaf growth stage, the 0.2-0.4 and 0.4-0.7 m layers at the silking growth stage (Figure 5), and the 0-0.2 m layer at the blister growth stage and from the milking growth stage to the dough growth stage. Overall, these data indicate that in a dry year with insufficient soil moisture, moisture from the 0-0.7 m soil layer contributes greatly to corn growth (93%). These results are inconsistent with the research findings of Ma and Song (2016), their research showed that the contribution of soil water of 0-0.9 m soil layer is 87%. The contribution of soil water is lower than our research results, and the main reason may be that the fertilizer we apply is organic fertilizer, while the fertilizer they apply is chemical fertilizer, and the organic fertilizer fully uses the upper soil moisture.

Mechanisms of organic fertilizer in enhancing soil water retention and regulating crop water uptake, and its interaction with rainfall distribution

Long-term application of organic fertilizer can significantly improve soil water retention capacity, increase water storage in the root zone, thereby promoting crop water uptake, reducing deep percolation, and ultimately enhancing water use efficiency (Liu et al., 2013; Zheng et al., 2017). Observations during the 2020 maize growing season (characterized by seasonal drought and no irrigation) in this study validated this mechanism and revealed important interactive effects between fertilization treatments and rainfall distribution. Specifically, in the shallow 0-0.2 m layer, the soil water content was lower under the organic fertilizer treatment (T2) (T2: 35%, T1: 44%), indicating stronger water uptake by crops from this layer. Conversely, in the deeper 0.7-1.2 m layer, the water content was higher under T2 (T2: 5%, T1: 10%), suggesting less water extraction. This indicates that in dry years, organic fertilizer application promoted maize to rely more on shallow soil water during the early (6^{th} leaf to 13^{th} leaf) and late (blister to dough stage) growth periods. In contrast, chemical fertilizer application alone (T1) led to greater dependence on deep soil water during the middle growth period (tasseling to silking stage). These results are consistent with previous research (Liu et al., 2013). The underlying mechanism is that organic fertilizer improves the physical structure of shallow soil by reducing bulk density, increasing the quantity of aggregates and soil porosity, thereby enhancing its water-holding capacity and retaining more water in the root zone for crop uptake (Zheng et al., 2017; Yang et al., 2018; Panday et al., 2024).

Rainfall distribution is a key environmental factor regulating the aforementioned water uptake patterns. In this study, two low-rainfall periods (late July to mid-August, and late August to late September) coincided with key maize growth stages from tasseling to dough stage. Analyses using stable hydrogen and oxygen isotopes (δD and $\delta^{18}O$) and the MixSIAR model revealed that during the first dry period (24 July-15 August, covering tasseling to silking), soil moisture in the shallow layer (0-0.4 m) decreased rapidly due to high evapotranspiration demand and insufficient recharge, forcing maize to increase its reliance on deeper water sources. For instance, the contribution rate of the 0.7-1.2 m soil layer was higher in the T1 treatment (10%) than in T2 (5%). The second dry period (29 August-22 September, covering milk to dough stage) further exacerbated the depletion of surface soil moisture, leading to an even more pronounced dependence on deep soil water (e.g., 0.6-1.2 m), as also evidenced by shifts in the $\delta^{18}O$ values of stem water.

Fertilization treatments significantly modulated crop responses to drought. The organic fertilizer treatment (T2), by improving aggregate stability and pore structure, enhanced the soil's water retention and buffering capacity. This allowed maize under T2 to maintain relatively high utilization of shallow water (with an average contribution rate of 44% from the 0-0.2 m layer) even during dry spells. In contrast, the chemical fertilizer treatment (T1) resulted in faster water loss from the soil during drought, forcing crops to extract more water from deeper layers (with a contribution rate of 10% from the 0.7-1.2 m layer), indicating a weaker buffering capacity against surface drying.

In conclusion, this study demonstrates that organic fertilizer primarily enhances water retention capacity by improving the physical structure of shallow soil, thereby optimizing crop water uptake patterns during seasonal drought. It promotes greater use of shallow water storage and reduces dependence on deep water. This mechanism interacts significantly with rainfall distribution. During critical dry growth periods, the water retention effect of organic fertilizer effectively buffers water stress and reduces the crop's need to shift to deep water uptake. These findings deepen the understanding of maize water uptake mechanisms in semi-arid regions and provide a theoretical basis for improving agricultural water use efficiency through organic amendments combined with water management. Future research should focus on how to utilize such measures to optimize irrigation scheduling under long-term climate change to adapt to erratic rainfall patterns.

Limitations of isotope techniques and model applications

In this work, we used the MixSIAR to quantify the contribution of water uptake from different soil depths. Of course, there is a certain degree of uncertainty in stem water extraction and water source apportionment based on the MixSIAR Bayesian mixing model. First, although cryogenic vacuum distillation is widely used to extract water from plant stems, isotopic fractionation may occur if the extraction efficiency is insufficient. Studies have noted that extraction yields below 95% could lead to deviations in δD and $\delta^{18}O$ values (West et al., 2006). In this study, we ensured extraction efficiency exceeded 98% by optimizing distillation time and temperature, minimizing this uncertainty. Second, soil water isotopic composition varies spatially and temporally due to evaporation, infiltration, and root water uptake preferences. The MixSIAR model accounts for such variability by incorporating standard deviations of source isotopes, but insufficient sampling density may still lead to misestimation of source contributions. Nevertheless, future work could reduce uncertainty by increasing isotopic sampling frequency, combining multiple tracers (e.g., soil water chemistry), or comparing with independent methods like root excavation.

CONCLUSIONS

We studied water uptake by corn from different soil layers at various growth stages to investigate its impact of the long-term different fertilizer treatments. Drought promoted the uptake of water from the shallow soil layer (0-0.7 m). The average contribution of soil water from the 0-0.2, 0.2-0.4, 0.4-0.7, and 0.7-1.2 m layers was 40%, 23%, 30%, and 7% during the experimental period, respectively. The water taken up was mainly sourced from depths of 0-0.7 m at the 9th growth stage (86%), 13th growth stage (91%), tassel growth stage (89%), silking growth stage (87%), blister growth stage (95%), and dough growth stage (94%); from 0-0.4 m at the 6th growth stage (85%); and from 0-0.2 m at the milk growth stage (81%). The two fertilization treatments led to clear differences in the proportional contribution of soil water, with more water from the 0-0.2 m soil layer consumed after the organic treatment. This might have been because the manure fertilizer improved the soil physical properties, leading to better water-holding capacity and less evaporation from the soil. These results on water uptake patterns and response mechanisms provide accurate and locally adapted information regarding manure fertilizer practices in northern China.

This study clarified the water uptake characteristics of corn after applying organic fertilizers, and providing a theoretical basis for developing a reasonable fertilization plan combining inorganic and organic fertilizers in arid areas. At the same time, by studying the water uptake levels of corn, the optimal depth of the water absorbing soil layer for corn can be determined, which has certain significance for the application of water-saving measures such as underground drip irrigation in the future. However, the results of this experiment only described the water uptake pattern of corn in dry years, and did not take into account the wet and normal precipitation years.

Author contributions

Conceptualization: Y.K-H. Methodology: J.H-H. Software: J.H-H. Validation: M.J-Z. Formal analysis: M.J-Z. Investigation: L.H-L. Resources: L.H-L. Data curation: J.H-H. Writing-original draft: L.H-L. Writing-review & editing: Y.K-H. Visualization: M.J-Z. Supervision: Y.K-H. Project administration: J.T-Z. Funding acquisition: J.T-Z. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

This work was partially supported by National Key Research and Development Program Project (Grant No. 2017YFD0300203).

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