Supporting Information for ***Environmental Science & Technology***

On-Site Treatment of Shale Gas Flowback and Produced Water in Sichuan Basin by Fertilizer Drawn Forward Osmosis for Irrigation

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S1. Water quality of FS and DS in FDFO system

In 2016, a total of 6 and 131 million m^3 of FPW was produced in China and USA, respectively and it is expected that this value will reach up to 50−55 and 499−3585 million m^3 in 2030 in China and USA, respectively.[1,2] In fact, the water quality exhibits obvious spatial and temporal changes.[3] The TDS of shale gas FPW varies significantly, ranging from 6906 to 28,900 $mg \cdot L^{-1}$ in Sichuan Basin, and more than 300,000 $mg \cdot L^{-1}$ was also reported in Marcellus, USA.[4] The goal of this study is to recycle or reuse the shale gas FPW to achieve the sustainable water reuse and shale gas development.

The FDFO has been assessed in many fields, such as sewage, mine impaired water and coal seam gas (CSG) produced water. As summarized in Table S1, compared to other types of feedwater used as FS in FDFO systems, the shale gas FPW in Sichuan Basin in this study is more complicated with higher salinity and relative low organics. A low feed concentration is more favorable in FDFO system. Usually, the diluted fertilizer DS still required substantial dilution to meet the irrigation water quality standards in terms of nutrient concentration,[5] and a large dilution factor may be required for final fertilizer solution when feedwater with a high TDS is used as FS. Some researchers have investigated hybrid processes for simultaneous wastewater treatment and the agricultural application, such as FDFO-nanofiltration,[6,7] and reverse osmosis-FDFO for dilution of fertilizers.[8] However, considering the utilization of solid/liquid fertilizer for agriculture irrigation, the nutrient concentrations obtained draw solution (DS) during FDFO operation are much lower than commercial solid/liquid fertilizers. Although a large amount of freshwater is needed during irrigation, a concentrated fertilizer solution is still beneficial to irrigation. Therefore, the salinity of shale gas FPW is not a limiting

factor for the utilization of FDFO for irrigation.

Table S2 summarizes the characteristics of the selected fertilizers as DS in FDFO system. The water extraction capacity of each fertilizer depends on the TDS of the FS, the solubility and molecular weight of the fertilizer, as well as the molar concentration of the fertilizer solution at osmotic equilibrium with the bulk osmotic pressure of the FS.[8]

Table S1 Characteristics of primary water quality parameters of feed solution (FS) used in this study and in literature using FDFO for irrigation

Parameter	Shale gas FPW	Raw sewage[9]	Mine impaired water[6]	CSG reverse osmosis brine[8]
Turbidity (NTU)	0.16±0.03	/	1.0±0.15	1
DOC ($mg \cdot L^{-1}$)	13.84±0.35	72.6±1.2	2.1±0.53	/
UV_{254} (cm^{-1})	0.053±0.005	/	/	/
pH	7.39±0.08	7.13±0.2	7.8±0.3	9.07
EC ($mS \cdot cm^{-1}$)	36.34±0.18	1.079±0.005	5.4±0.5	22.58±0.02
TDS ($mg \cdot L^{-1}$)	22,530±120	/	2491±85	15,354±12
Li^+ ($mg \cdot L^{-1}$)	39.54±7.56	/	/	/
Na^+ ($mg \cdot L^{-1}$)	8350±205	/	812±67	6089±48
K^+ ($mg \cdot L^{-1}$)	139.6±4.1	18.2±1.1	7.0±1.1	28.7±0.6
NH_4^+ ($mg \cdot L^{-1}$)	124.2±15.7	38.5±5.8	12.0±4.0	/
Ca^{2+} ($mg \cdot L^{-1}$)	429.4±19.0	/	48.0±3.8	36.3±0.6
Mg^{2+} ($mg \cdot L^{-1}$)	50.69±7.32	/	22.0±2.1	14.7±0.6
Mn^{2+} ($mg \cdot L^{-1}$)	54.18±5.62	/	0.01	/
Sr^{2+} ($mg \cdot L^{-1}$)	71.13±7.98	/	/	/
F^- ($mg \cdot L^{-1}$)	7.37±1.96	/	/	/
Cl^- ($mg \cdot L^{-1}$)	13020±463	/	983±26	4793±87
Br^- ($mg \cdot L^{-1}$)	102.4±14.3	/	/	/
NO_3^- ($mg \cdot L^{-1}$)	29.94±6.67	/	< 0.005	/
SO_4^{2-} ($mg \cdot L^{-1}$)	19.71±3.64	/	607±27	23.3±3.1
Si ($mg \cdot L^{-1}$)	/	5.4 ±0.5	< 0.009	5.21±0.17
SAR	101.3±4.9	/	/	215.3±1.2
π (bar)	17.4	/	/	/

Note: DOC, dissolved organic carbon; EC, electrical conductivity; TDS, total dissolved solid; SAR, sodium adsorption ration; π, osmotic pressure.

Table S2 Characteristics of the selected fertilizers as DS in FDFO system

Fertilizers	pH [a]	EC ($mS \cdot cm^{-1}$) [a]	Solubility ($mol \cdot L^{-1}$) [b]	Osmotic pressure (bar) [b]	CAS number	Purity (%)	Supplier
KCl	5.15	204	4.6	89.3	7447-40-7	99.5	Kelong
SOA	5.20	226	5.7	92.1	7783-20-2	99.0	Chemical
MAP	3.75	83.65	3.7	86.3	7772-76-1	99.0	(Chengdu,
DAP	8.33	102.8	6.5	95.0	7783-28-0	99.0	China)
KNO_3	5.31	156.9	3.3	64.9	7757-79-1	99.0	
	MW ($g \cdot mol^{-1}$)	Concentration at 17.4 bar ($mol \cdot L^{-1}$)	Volume from FPW ($L \cdot kg^{-1}$) [c]	Species ($mol \cdot L^{-1}$) [b]			
KCl	74.6	0.413	26.6	K^+, 1.99; Cl^-, 1.99; KCl (aq.), 0.01.			
SOA	132.1	0.361	18.5	NH_4^+, 3.07; SO_4^{2-}, 1.07; $NH_4SO_4^-$, 0.93.			
MAP	115.0	0.392	16.1	NH_4^+, 2.0; $H_2PO_4^-$, 1.76; $H_2P_2O_7^{2-}$ (ion), 0.10; H_3PO_4 (aq.), 0.02; $HP_2O_7^{3-}$ (ion), 0.004.			
DAP	132.1	0.316	21.7	NH_4^+, 3.94; HPO_4^{2-} (ion), 1.79; $P_2O_7^{4-}$ (ion), 0.07; $H_2PO_4^-$ (ion), 0.02; $HP_2O_7^{3-}$ (ion), 0.02.			
KNO_3	101.1	0.413	17.4	K^+, 2.0; NO_3^-, 2.0.			

Note: a. Values were measured using 2 $mol \cdot L^{-1}$ of DS at 25°C; b. Values were obtained at a temperature of 25°C;[10] c. Water extraction capacity was calculated using the equation modified with SRSF.[8]

S2. Feed concentration factor and characteristics of FO membranes after operation

When shale gas FPW was used as FS, the variations of EC in FS with operation time using different fertilizer DS are illustrated in Figure S1. The EC increased fastest for DS using KCl, followed by KNO_3, SOA and MAP, while the slowest increase was observed for DAP.

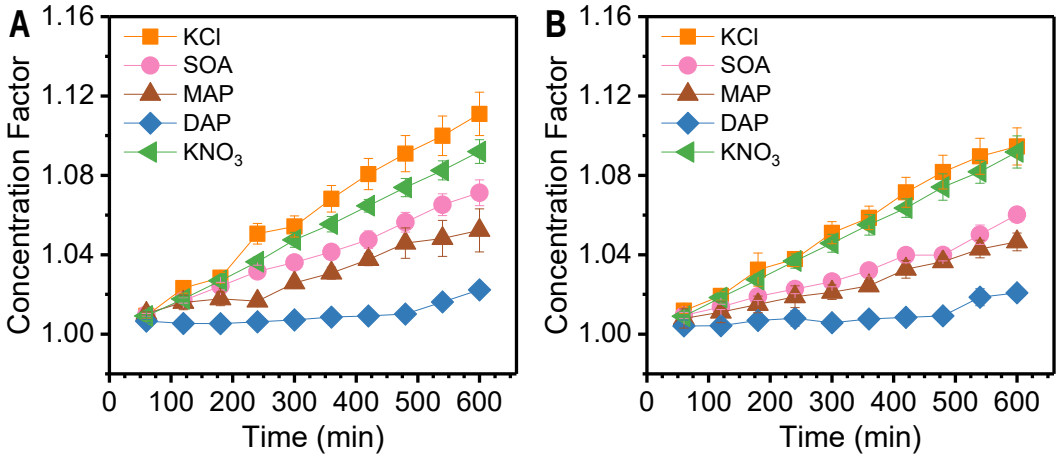

Figure S1. Variation of electrical conductivity in FS with operation time: (A) under the AL-DS mode and (B) under the AL-FS mode.

The SEM images of virgin membranes, fouled membranes and membranes after osmotic backwashing using different types of fertilizers are presented in Figure S2. An energy dispersive spectrometer (EDS, X-Max Extreme, Oxford-Instruments) that was equipped with SEM was used to detect the elemental compounds of membrane surface at a magnification of 2000. The EDS analysis for virgin and fouled FO membranes using different types of fertilizers as DS is summarized in Figure S3. Scaling powder in fouled membrane using DAP as DS under AL-DS mode was collected and was analyzed by PANalytical Empyrean X-ray diffraction (XRD) with 2θ ranging from 10° to 70° (Cu Kα, λ=1.540598 Å), as shown in Figure S4.

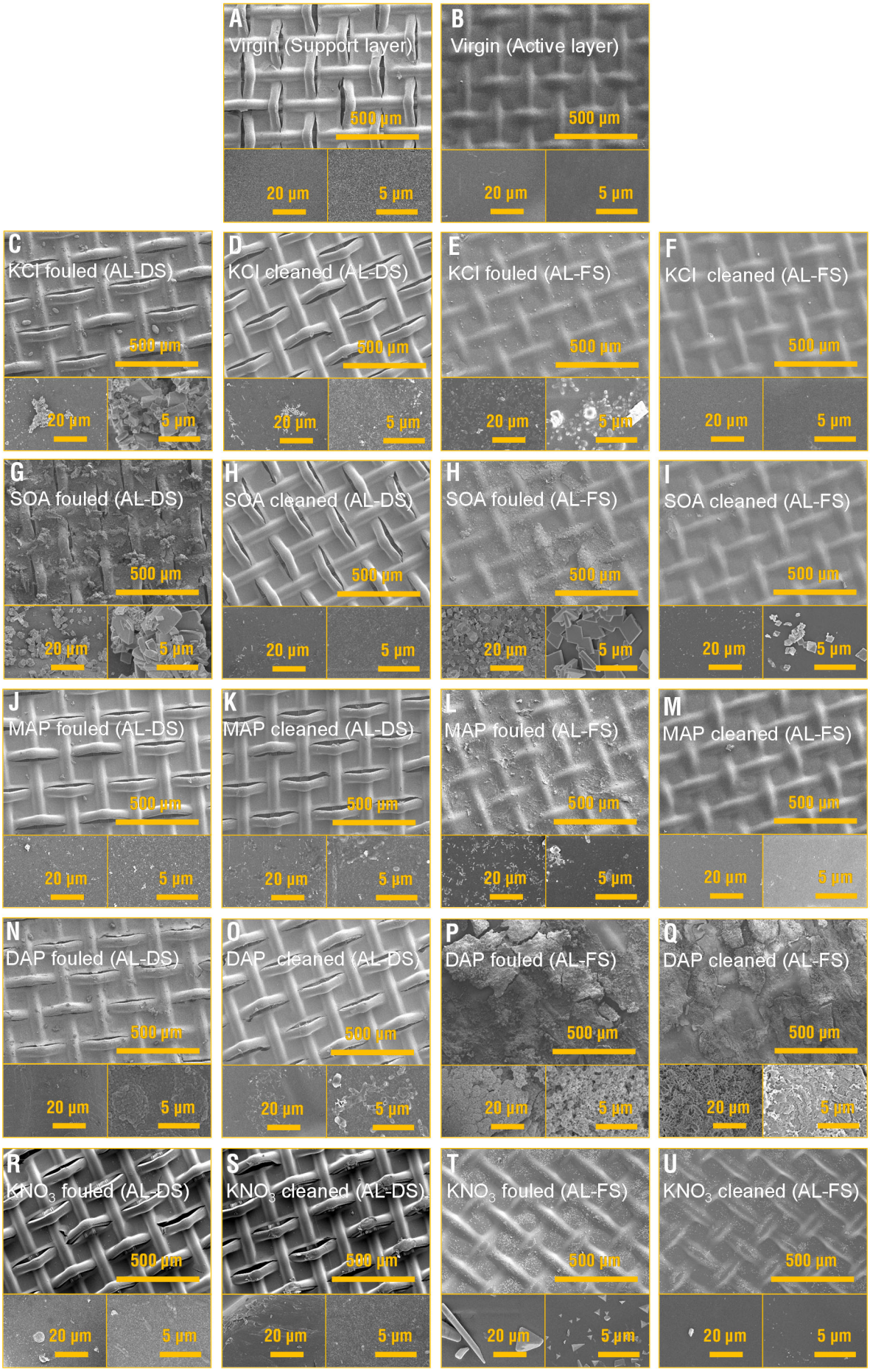
A
Virgin (Support layer)
500 µm
20 µm
5 µm
B
Virgin (Active layer)
500 µm
20 µm
5 µm
C
KCl fouled (AL-DS)
500 µm
20 µm
5 µm
D
KCl cleaned (AL-DS)
500 µm
20 µm
5 µm
E
KCl fouled (AL-FS)
500 µm
20 µm
5 µm
F
KCl cleaned (AL-FS)
500 µm
20 µm
5 µm
G
SOA fouled (AL-DS)
500 µm
20 µm
5 µm
H
SOA cleaned (AL-DS)
500 µm
20 µm
5 µm
H
SOA fouled (AL-FS)
500 µm
20 µm
5 µm
I
SOA cleaned (AL-FS)
500 µm
20 µm
5 µm
J
MAP fouled (AL-DS)
500 µm
20 µm
5 µm
K
MAP cleaned (AL-DS)
500 µm
20 µm
5 µm
L
MAP fouled (AL-FS)
500 µm
20 µm
5 µm
M
MAP cleaned (AL-FS)
500 µm
20 µm
5 µm
N
DAP fouled (AL-DS)
500 µm
20 µm
5 µm
O
DAP cleaned (AL-DS)
500 µm
20 µm
5 µm
P
DAP fouled (AL-FS)
500 µm
20 µm
5 µm
Q
DAP cleaned (AL-FS)
500 µm
20 µm
5 µm
R
KNO3 fouled (AL-DS)
500 µm
20 µm
5 µm
S
KNO3 cleaned (AL-DS)
500 µm
20 µm
5 µm
T
KNO3 fouled (AL-FS)
500 µm
20 µm
5 µm
U
KNO3 cleaned (AL-FS)
500 µm
20 µm
5 µm

Figure S2. Comparison of SEM images of (A)(B) virgin membranes, (C-U) membranes after fouling and after osmotic backwashing for FDFO using different types of fertilizers under AL-DS and under AL-FS.

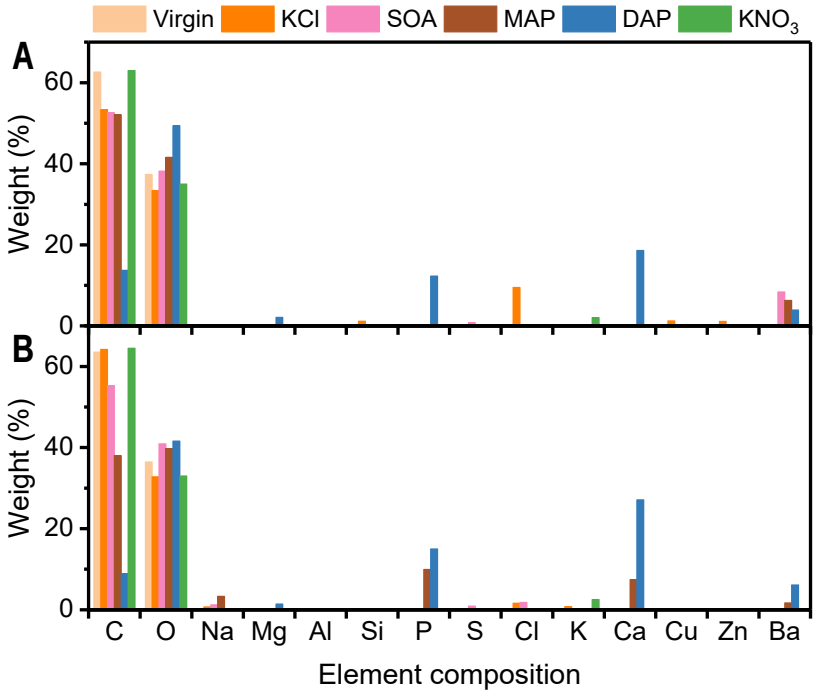

Figure S3. Element composition of virgin and fouled FO membranes by EDS analysis using different types of fertilizers (A) under the AL-DS mode and (B) under the AL-FS mode.

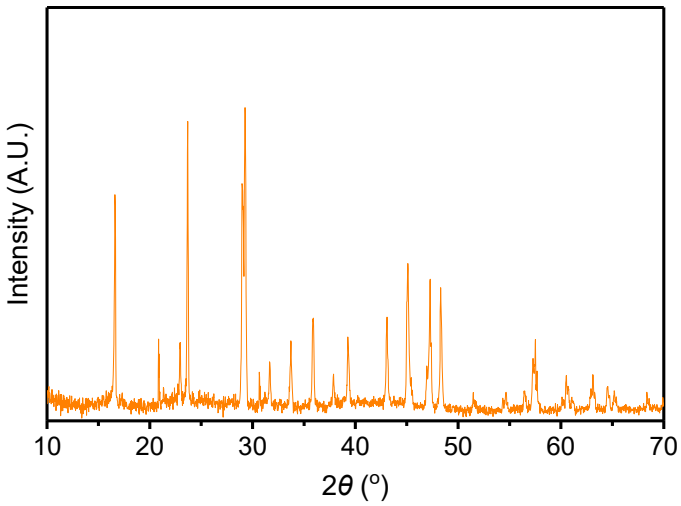

Figure S4. XRD pattern of fouled membrane using DAP as DS under AL-DS mode. The XRD peaks agree well with those of commercial struvite.[11]

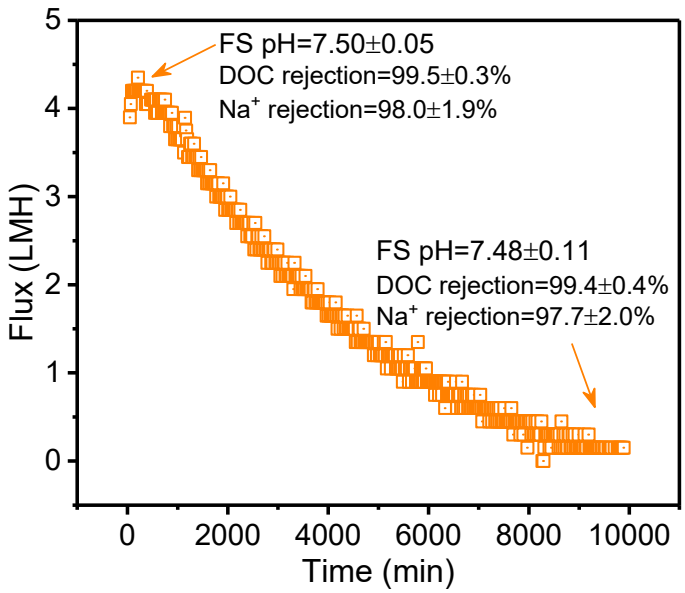

Figure S5. Variation in permeate flux with time in long-term operation of FDFO using mixed fertilizers as DS. The pH of feed solution (FS), rejections of DOC and Na^+ are labeled in the figure. Experimental conditions: Shale gas FPW as FS, mixed fertilizers of KNO_3 (1 $mol \cdot L^{-1}$) and MAP (1 $mol \cdot L^{-1}$) as DS; crossflow velocity of 8.3 $cm \cdot s^{-1}$; temperature of 25 ± 0.5 °C; water recovery of 35%.

S3. Culture of Chinese cabbage in soil

To further investigate the effect of irrigation water on the physiological characteristics of plants, the Chinese cabbages were also cultivated in soil for 8 weeks. The seeds were grown in rectangle tabletop planters with the effective height, width and length of 14, 20 and 60 cm, respectively. Five groups were cultivated for each type of irrigation water, with 5 seeds for each group, and an apart distance of approximately 10 cm was kept for each group. All groups were subjected to irrigation every day by different types of irrigation water.

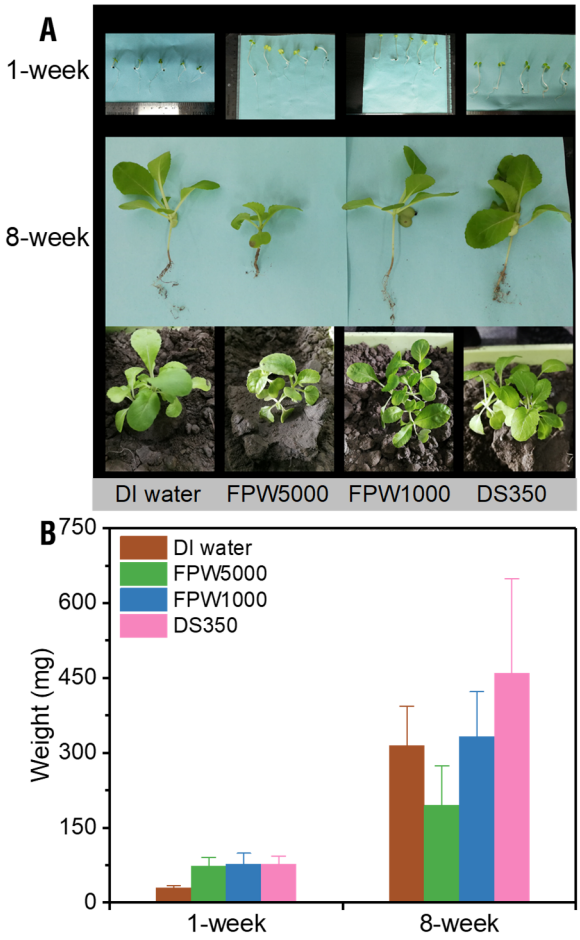

Figure S6. (A) Photos of Chinese cabbage and (B) variation of the weight of each Chinese cabbage with time using different types of irrigation water. In chart B, the increase in weight (i.e., the difference of final weight and seed weight) was used as y-axis.

S4. RNA extraction and gene expression

RNA extraction. The total RNA of whole plant was extracted using TRIzol® Reagent (Invitrogen) following the manufacturer's guidelines and genomic DNA was removed using DNase I (TaKara). Then RNA quality was determined by 2100 Bioanalyser (Agilent) and quantified using a NanoDrop2000 (Vernon Hills, IL, USA) spectrophotometer at wavelengths of 230, 260 and 280 nm. Only high-quality RNA sample ($OD_{260/280}$ = 1.8~2.2, $OD_{260/230} \geq 2.0$, RIN ≥ 6.5, 28S/18S ≥ 1.0, content > 2 μg) was used to construct sequencing library.

Library preparation, and Illumina Hiseq novaseq6000 Sequencing. RNA-seq transcriptome librariy was prepared following TruSeqTM RNA sample preparation Kit from Illumina (San Diego, CA) using 1 μg of total RNA. Shortly, messenger RNA was isolated according to polyA selection method by oligo(dT) beads and then fragmented by fragmentation buffer firstly. Secondly double-stranded cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA) with random hexamer primers (Illumina). Then the synthesized cDNA was subjected to end-repair, phosphorylation and 'A' base addition according to Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 200–300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantified by TBS380, paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq xten (2 × 150 bp read length).

Read mapping. The raw paired end reads were trimmed and quality controlled by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) with default parameters. Then clean reads were separately aligned to reference genome with

orientation mode using TopHat (http://tophat.cbcb.umd.edu/, version 2.0.0) software.[12] The mapping criteria of bowtie was as follows: sequencing reads should be uniquely matched to the genome allowing up to 2 mismatches, without insertions or deletions. Then the region of gene was expanded following depths of sites and the operon was obtained. In addition, the whole genome was split into multiple 15 kbp windows that share 5 kbp. New transcribed regions were defined as more than 2 consecutive windows without overlapped region of gene, where at least 2 reads mapped per window in the same orientation.

Differential expression analysis and Functional enrichment. To identify DEGs (differently expressed genes) between two different samples, the expression level of each transcript was calculated according to the fragments per kilobase of exon per million mapped reads (FRKM) method. RSEM (http://deweylab.biostat.wisc.edu/rsem/)[13] was used to quantify gene abundances. R statistical package software EdgeR (Empirical analysis of Digital Gene Expression in R, (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html)[14] was utilized for differential expression analysis. In addition, functional-enrichment analysis including GO and KEGG were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at Bonferroni-corrected P-value $\leq$0.05 compared with the whole-transcriptome background. GO functional enrichment and KEGG pathway analysis were carried out by Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do).[15]

Table S3 Quality of RNA samples for Cherry radish and Chinese cabbage using different types of irrigation water

Irrigation water	Concentration ($ng \cdot \mu L^{-1}$)	Content (μg)	OD_{260}/OD_{280}	OD_{260}/OD_{230}	28S/18S	RNA Number	Integrity
Cherry radish							
DI water	2479.9	86.80	2.11	2.29	1.6	8.6	
FPW5000	2319.5	81.18	2.13	2.27	1.6	8.9	
FPW1000	1999.7	69.99	2.17	2.18	1.9	8.9	
DS350	2386.0	83.51	2.11	2.25	1.6	9.0	
Chinese cabbage							
DI water	1808.0	63.28	2.17	2.31	1.7	8.7	
FPW5000	758.3	26.54	2.19	2.3	1.9	8.2	
FPW1000	801.8	28.06	2.21	2.29	1.7	8.0	
DS350	1069.1	37.42	2.20	2.32	1.6	8.2	

Table S4 The detailed information of sequenced data for Cherry radish and Chinese cabbage

Irrigation water	Raw reads	Clean reads	%≥Q30	Mapped reads	Multiple mapped	Uniquely mapped
Cherry radish						
DI water	44,338,788	43,917,464	94.11%	35,254,734 (80.27%)	7.91%	72.36%
FPW5000	49,561,634	48,986,252	92.38%	39,349,305 (80.33%)	8.00%	72.33%
FPW1000	44,565,520	44,165,076	93.80%	35,832,188 (81.13%)	8.60%	72.53%
DS350	52,143,180	51,664,242	93.84%	41,884,443 (81.07%)	8.33%	72.74%
Chinese cabbage						
DI water	53,426,728	52,831,954	92.27%	47,034,412 (89.03%)	2.58%	86.44%
FPW5000	51,374,066	50,875,756	93.14%	45,276,031 (88.99%)	2.94%	86.06%
FPW1000	52,298,708	51,734,182	93.12%	45,876,940 (88.68%)	3.05%	85.63%
DS350	43,824,200	43,317,844	92.60%	38,453,195 (88.77%)	2.67%	86.10%

Table S5 Derivation distribution of the mapped reads for Cherry radish and Chinese cabbage

Irrigation water	Introns	3'UTR	CDS	5'UTR	Intergenic
Cherry radish					
DI water	504324.0 (0.94%)	2129100.0 (3.96%)	48691498.0 (90.60%)	2085154.0 (3.88%)	333732.0 (0.62%)
FPW5000	446233.0 (0.74%)	2002980.0 (3.3%)	55705550.0 (91.90%)	2135714.0 (3.52%)	327411.0 (0.54%)
FPW1000	441516.0 (0.81%)	1914922.0 (3.52%)	49541618.0 (91.09%)	2113513.0 (3.89%)	376152.0 (0.69%)
DS350	519022.0 (0.8%)	2092957.0 (3.24%)	59140808.0 (91.53%)	2508165.0 (3.88%)	355181.0 (0.55%)
Chinese cabbage					
DI water	1527386.0 (2.31%)	0.0 (0.0%)	63175059.0 (95.55%)	0.0 (0.0%)	1411948.0 (2.14%)
FPW5000	1360634.0 (2.13%)	0.0 (0.0%)	61184407.0 (95.70%)	0.0 (0.0%)	1387399.0 (2.17%)
FPW1000	1391722.0 (2.17%)	0.0 (0.0%)	61257325.0 (95.67%)	0.0 (0.0%)	1381645.0 (2.16%)
DS350	1184041.0 (2.18%)	0.0 (0.0%)	51976003.0 (95.65%)	0.0 (0.0%)	1179426.0 (2.17%)

Note: CSD, coding sequence; UTR, untranslated region.

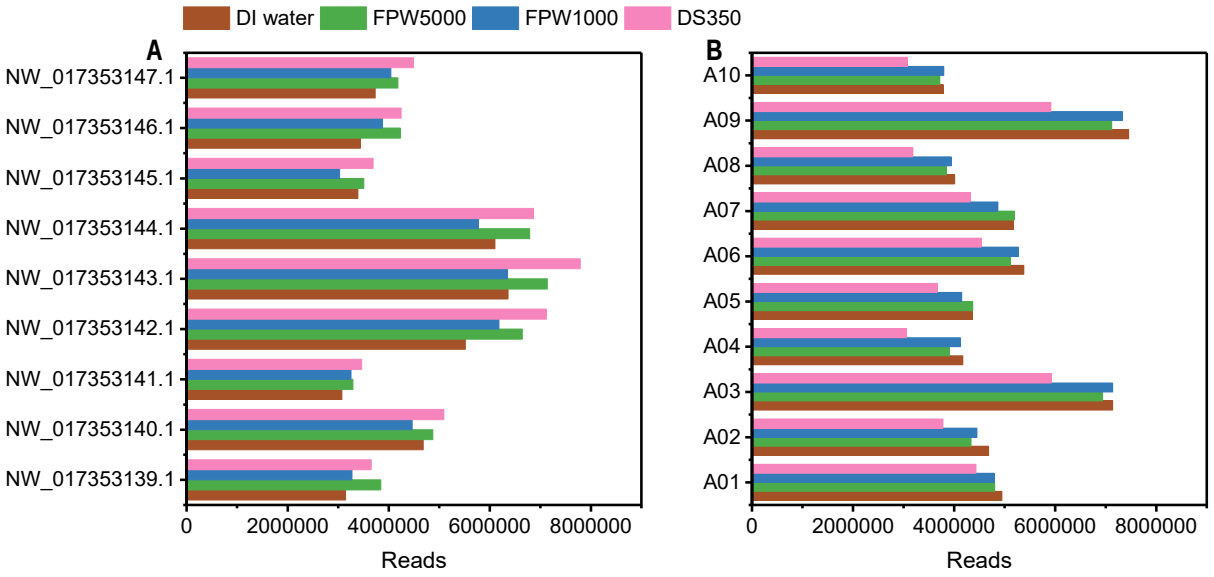

Figure S7. The chromosome distribution of the mapped reads (top 10) using different types of irrigation water: (A) Cherry radish and (B) Chinese cabbage.

Table S6 Correlation between expressed genes of samples for Cherry radish and Chinese cabbage

Irrigation water	DI water	FPW3000	FPW1000	DS350
Cherry radish				
DI water	1.000	0.945	0.941	0.945
FPW3000	0.945	1.000	0.950	0.949
FPW1000	0.941	0.950	1.000	0.945
DS350	0.945	0.949	0.945	1.000
Chinese cabbage				
DI water	1.000	0.925	0.942	0.912
FPW3000	0.925	1.000	0.967	0.940
FPW1000	0.942	0.967	1.000	0.959
DS350	0.912	0.940	0.959	1.000

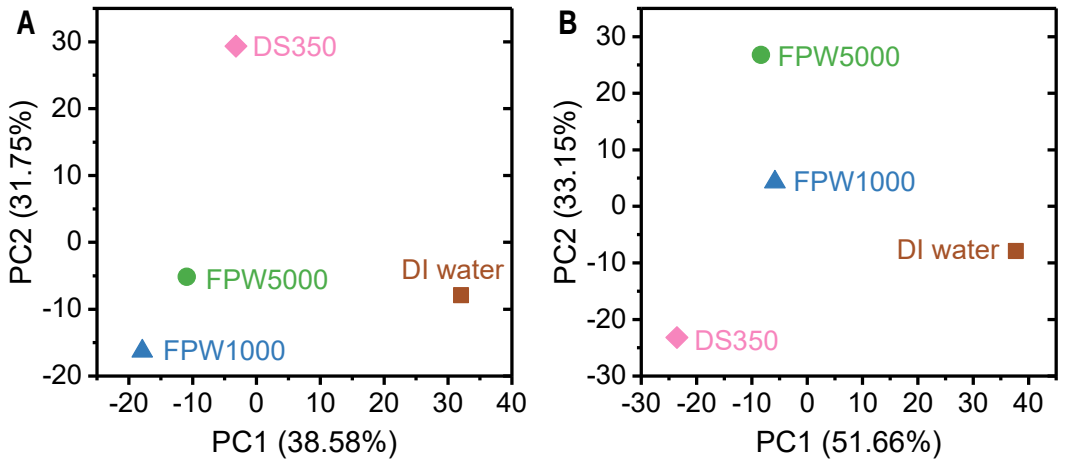

Figure S8. Principal component analysis (PCA) for expressed genes of (A) Cherry radish and (B) Chinese radish using different types of irrigation water resources.

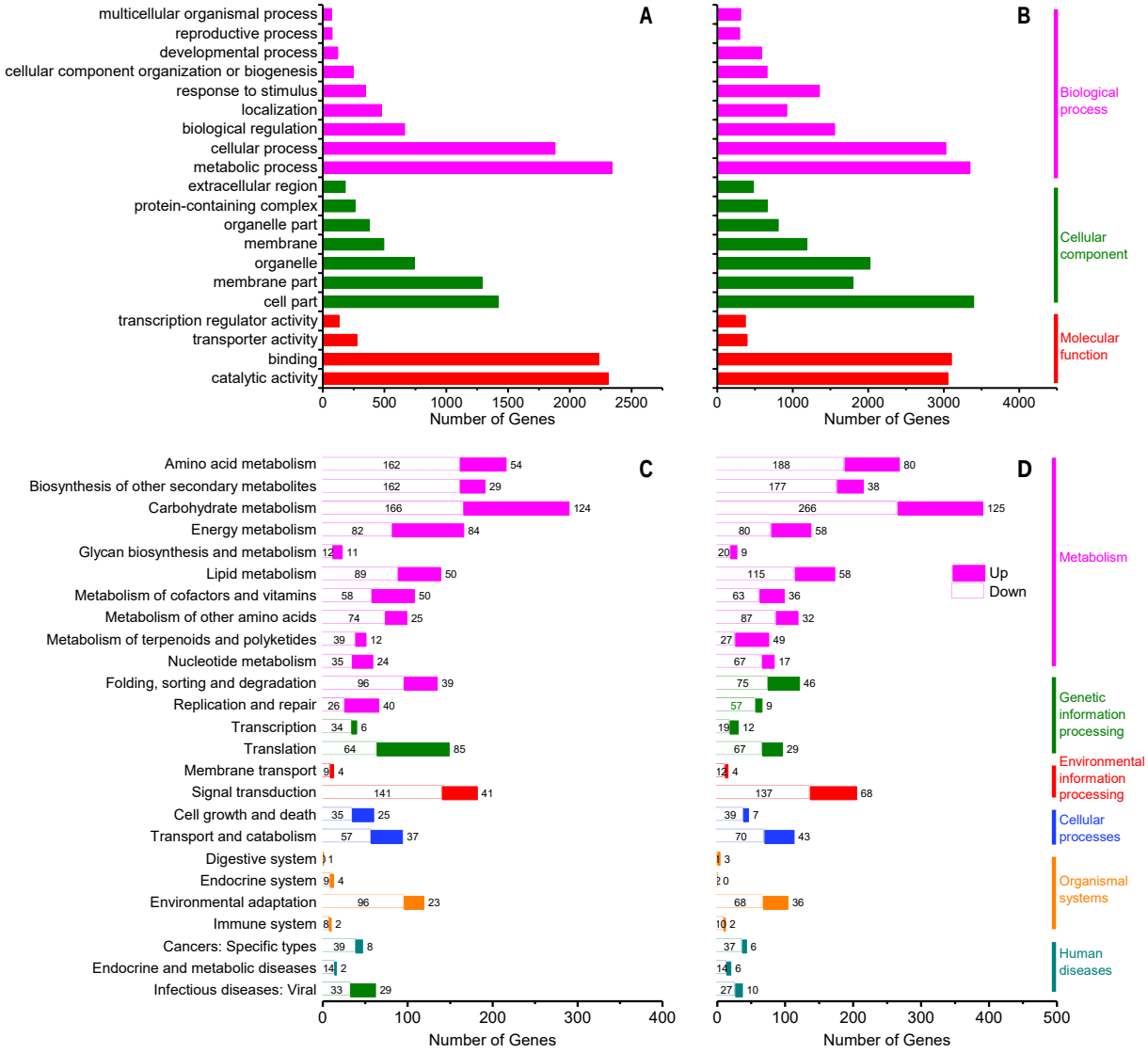

Figure S9. Function analysis of the intersection DEGs: (A),(B) GO annotation analysis and

(C),(D) KEGG annotation analysis of the DEGs between (A),(C) cherry radish and (B),(D) Chinese cabbage irrigated with DI water and FPW5000.

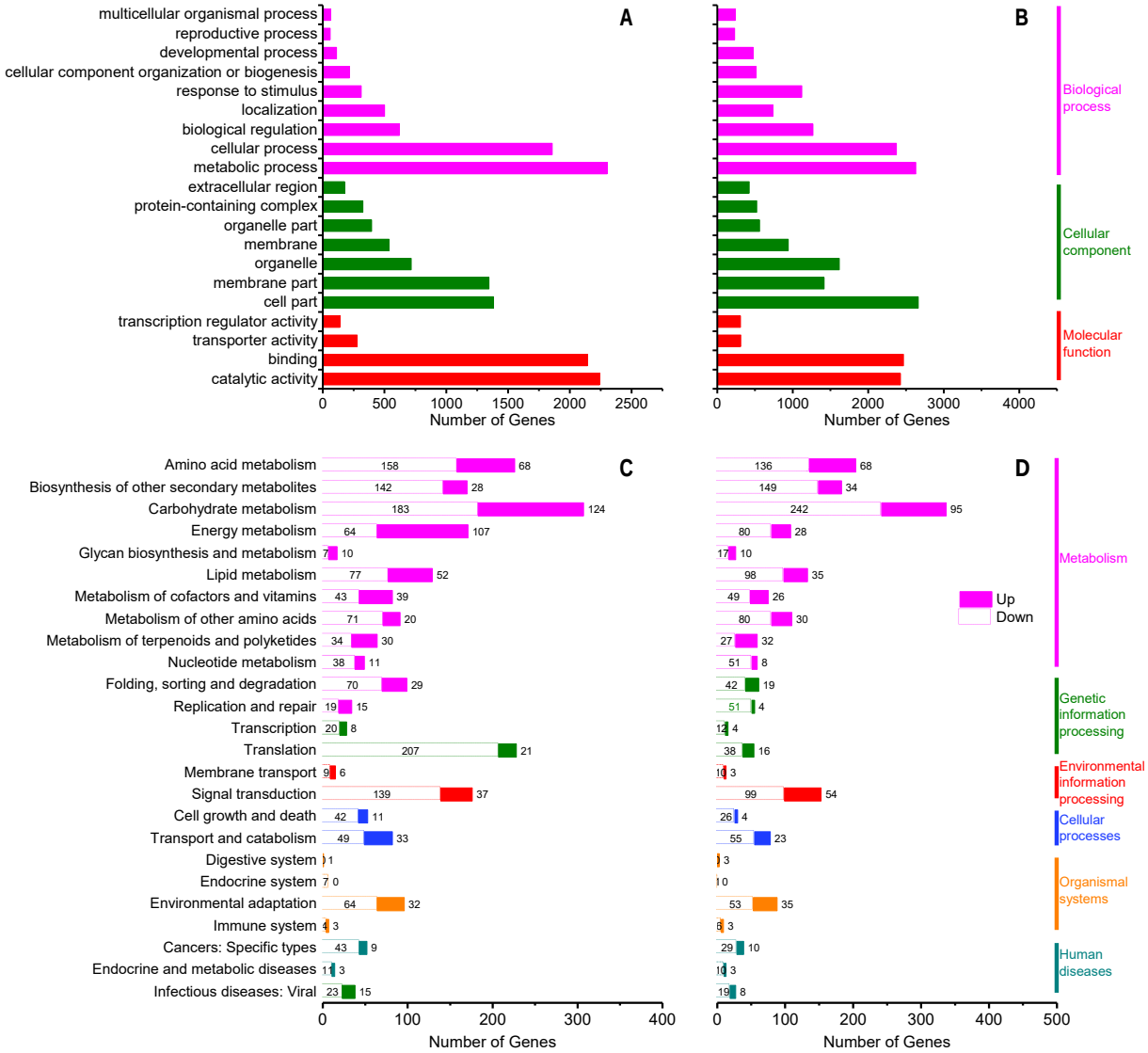

Figure S10. Function analysis of the intersection DEGs: (A),(B) GO annotation analysis and (C),(D) KEGG annotation analysis of the DEGs between (A),(C) cherry radish and (B),(D) Chinese cabbage irrigated with DI water and FPW1000.

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