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Influence of Temperature and Vegetation on Selenium Removal in Constructed Wetland Microcosms[1]

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Abstract. Intense precipitation events over coal fly ash sediments produce large runoff volumes that can accumulate and transport selenium (Se) into surrounding watersheds and degrade the quality of aquatic ecosystems. Constructed wetland (CW) phytoremediation is a cost-effective, ecological water treatment alternative that relies on plant metabolic processes to improve the quality of Se-impacted runoff. This research was conducted to evaluate the seasonal influence on Se removal in simulated CWs planted with either cattail (CAT; *Typha angustifolia* L.) or duckweed (DWD; *Lemna minor* L.) compared to an unplanted (UNP) control over four consecutive, week-long flood events. CWs were simulated in 110 L microcosms containing 25 kg of Catalpa silty clay loam. Microcosms were acclimated for 14 d before the first flood event, when each received 30 L of simulated selenate-impacted runoff. Runoff treatments included 0, 1x or 2x Se rates. Water, plant, and soil samples were collected at application and six d after treatment application. Total Se concentration [Se] was determined with inductively coupled plasma mass spectrometry (ICP-MS). Data were analyzed with PROC MIXED (α=0.05). Overall, CAT and DWD significantly decreased aqueous [Se] by 47% compared to 36% for UNP ($P<0.0001$). Results indicate the greatest aqueous Se removal was observed in the summer (73%) followed by the fall (42%) and spring (41%). Temperature was strongly correlated with Se removal (0.65, $P<0.0001$). At temperatures between 15 and 20°C, CAT and DWD are suitable aquatic species for phytoremediation of selenate-impacted waters in CWs.

Additional Key Words: cattail (CAT), constructed wetlands (CWs), duckweed (DWD), phytoremediation, selenate.

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Introduction

In the United States, coal-fired energy supplies 65% of the nation's power demand. However, coal contains many harmful trace metals, and emissions must meet the requirements of the Clean Air Act Amendment (1990). Coal-fired power plants use flue gas desulfurization (FGD) technology to adsorb harmful trace elements to an alkaline material, typically limestone, producing fly ash. In Mississippi, combustion of surface-mined lignite raises concerns about potential downstream impacts of runoff over fly ash surfaces. Intense precipitation events produce runoff that can accumulate and transport Se into surrounding watersheds and degrade the quality of aquatic ecosystems. The National Pollutant Discharge Elimination System (NPDES) mandates [Se] in discharged runoff cannot exceed 11.8 ppb Se within a single discharge event (USEPA, 2016). Furthermore, monthly mean Se discharge concentrations cannot exceed 4.6 ppb Se (MDEQ, 2017a). Selenium-impacted runoff is typically detained in impoundments or settling ponds until NPDES standards are met. However, Mississippi Coal Mining Regulations (MCMR), Section 5377(f) stipulates 90% of the runoff shall be discharged within 10 d of rainfall (MDEQ, 2017b). Failure to meet NPDES and MDEQ standards constrains water supply and threatens the livelihood of entire ecosystems. To meet both federal and state regulations, stakeholders need a rapid, cost-effective mechanism to treat large runoff volumes. Constructed wetland (CW) phytoremediation may offer a potential augmentation to detention ponds for continuous, rapid abatement of Se-impacted runoff.

Selenium is a naturally occurring mineral present in many soils at concentrations ranging from 0.1 to 2 ppm (Fordyce, 2007). Selenium is an essential dietary mineral for humans and livestock. As a nutrient, Se is an essential constituent of selenocysteine and other selenoproteins stimulating improved immuno-response, thyroid activity, male fertility, and free radical detoxification in humans (Wu, 2004; Pilon-Smits and LeDuc, 2009; Rayman, 2012). Being the chemical analog to sulfur (S), non-specific substitution of Se for S in protein structures may exceed the narrow range between deficiency and toxicity (40-400 $\mu g\ d^{-1}$) (WHO, 1996). However, chronic Se exposure can lead to teratogenic and reproductive deformities in fish and waterfowl (Ohlendorf et al., 1986; Lemly, 2014). Aqueous Se toxicity thresholds vary by ecosystem based on Se chemical form, wildlife and fish species, food-web models, hydrology, etc. (Lemly, 1999). Bioavailable Se exists as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). Selenite could be considered more toxic to biota because SeO_3^{2-} is less reactive compared to SeO_4^{2-} and may increase the absorption rate. On the other hand,

SeO_4^{2-} is more bioavailable and can be difficult to remove from the environment because the rate limiting step for uptake is reduction to SeO_3^{2-} (Masscheleyn and Patrick, 1993; Zayed et al., 1998).

Constructed wetland (CW) phytoremediation may be a cost-effective, ecological alternative to conventional water treatment options to meet NPDES regulations. This "green" technology relies on a sustainable energy source (solar radiation) and renewable resources (plants) to improve runoff water quality. These systems require little capital to construct and minimal maintenance after completion (Frankenberger et al., 2004). CWs can be strategically designed for site specific management of stormwater runoff based on the needs of various industrial operations. Judicious selection of plant materials allows environmental managers to treat runoff or wastewaters impacted by several contaminants. Marchand et al. (2010) and Rezania et al. (2016) cite 61 research studies showing several plant species to effectively remove various trace metals and nutrients including, Al, As, Cr, Fe, Mn, N, P, Pb, Se, and Zn. While CWs provide food and habitat to a diverse community of species, overexposure can increase the risk of Se toxicity. Plant materials may reach a contaminant saturation threshold and require harvesting or replacement. However, there is potential for harvested materials to be sold as a feed supplement, green manure, or biofuel feedstock (Kaur et al., 2019).

CW phytoremediation capitalizes on biogeochemical and plant metabolic processes to remove a myriad of contaminants, not just Se, through three primary elimination pathways: rhizofiltration, phytoaccumulation, and phytovolatilization. Rhizofiltration concentrates Se on or near plant roots (Dushenkov et al., 1995). Phytoaccumulation occurs as plants transport and store Se in tissues via the S assimilatory pathway (Pilon-Smits and Quinn, 2010). Once inside the plant, Se is either stored in plant tissues or, potentially, methylated into volatile organic compounds that are released into the atmosphere, in a process known as phytovolatilization (Terry et al., 2000). These organoselenides are 600 times less toxic compared to inorganic Se compounds (Wilber, 1980). Because of this, phytovolatilization is an attractive elimination pathway in CWs as Se is completely removed from the ecosystem. Unfortunately, only a select population of terrestrial and aquatic plants are known to phytovolatilize inorganic Se.

Cattail (*Typha angustifolia* L.; CAT) is an emergent aquatic plant well adapted to wetland environments throughout much of the United States. Specialized aerenchyma tissue supply O_2 to soil microbes aiding microbial respiration. CAT remove Se through rhizofiltration, phytoaccumulation, and phytovolatilization (Pilon-Smits et al., 1999). When supplied with SeO_4^{2-}

at 19 ppb Se (inlet), CAT demonstrates moderate aqueous Se removal ranging from 45 to 65% of the applied Se (Gao et al., 2003; Lin and Terry, 2003; Shardendu et al., 2003; Nattrass et al., 2019). In CWs planted with CAT and supplied with 30 ppb Se as SeO_3^{2-}, Hansen et al. (1998) reported 89% Se mass removal and attributed 30% of the removal to volatilization (180 µg Se m^{-2} d^{-1}). A laboratory study showed CAT treated with 1600 ppb volatilized both Se chemical forms at a rate of 2 mg Se m^{-2} d^{-1} (Pilon-Smits et al., 1999).

Duckweed (*Lemna minor* L.; DWD) is a floating macrophyte known for its rapid vegetative reproduction that forms a dense mat over water surfaces creating a large root surface area. DWD roots remove aqueous Se primarily through rhizofiltration and, subsequently, plants phytoaccumulate Se (Allen, 1991; Zayed et al., 1998). In contrast to CAT, DWD can deplete the aqueous oxygen supply, potentially reducing bioavailable forms of Se to immobile forms. Miranda et al. (2014) reported DWD removed 55% of applied Se at aqueous concentrations of 83 ppb Se. When supplied with SeO_3^{2-} at concentrations ranging from 1 to 50 ppm, Carvalho and Martin (2001) observed nearly quantitative removal. Duckweed is gaining popularity as a feedstock for biofuel production and can be used for Se biofortification in livestock feed (Miranda et al., 2014).

One potential drawback to phytoremediation is nearly all treatment wetlands undergo seasonal cycling (Kadlec and Reddy, 2001). Plant metabolic processes are dependent upon abiotic factors such as temperature, photoperiod, and air flow, to name a few. Seasonal variation results from variation in internal oxygen transport and root respiration due to seasonal cycles of temperature, photoperiod, photosynthesis, stomatal activity, and nutrient consumption (Stein and Hook, 2003). Many studies have noted seasonal effects on the change in Se removal from large scale surface-flow CWs (Allen, 1991; Hansen et al., 1998; Gao et al., 2003). However, those studies were conducted in temperate climates of the western US.

A notable difference between this study and previous research is the CW design. Previous research conducted in surface flow CWs indicate little change between influent and effluent Se concentrations ([Se]), but rather determine success based on reduction in Se mass between influent and effluent (Hansen et al., 1998; Gao et al., 2003). Researchers noted concentration and dilution effects from evaporation and rainfall events along with temporal and spatial variability. Regulatory compliance is based on aqueous [Se] discharged into the watershed, not the overall change in Se mass. Therefore, a system that specifically achieves the desired decrease in aqueous [Se] is paramount. In our study, benchtop simulated CWs were used to evaluate the efficacy of detain-drain system supplied with SeO_4^{2-}-impacted runoff. The major advantages of our design are the ability to control environmental inputs or losses (i.e., precipitation or evapotranspiration), plant populations, and maintain a consistent flood level. This experiment was conducted outdoors in a small area (32 m^2) where much of the spatial variability that may exist within large field-scale CWs was controlled.

Objectives

CWs rely on solar radiation for plant growth and metabolism; thus, Se removal is expected to vary throughout the year. Therefore, the objective of this study was to determine temperature influence on Se removal efficiency in simulated CWs planted with either CAT or DWD compared to an unplanted (UNP) system over four consecutive, week-long flood events.

Materials and Methods

This research was conducted at Mississippi State University (33° 28' 9" N; 88° 47' W) from April 2018 to February 2019. Each four-week evaluation period began 21 d following an equinox or solstice event and continued for 28 d (Allen, 1991; Allen et al., 2002). CWs were simulated in 110 L containers with 25 kg of air-dried Catalpa silty clay loam. To minimize pore space variability, soil was passed through a 1.27 cm mesh screen. All CAT plants were collected from the same ramet and roots trimmed to a uniform size. Duckweed was collected from a pond located at (33° 25' 33" N, 88° 43' W). Microcosms were planted with either four CAT shoots trimmed to uniform height of 46 cm or 250 g of fresh DWD. UNP microcosms served as a negative control. Prior to Se application, microcosms were flooded with municipal water to a depth of 11 cm (30 L) and acclimated for 14 d. Municipal water [Se] was below the detectable limit (0.33 ppb Se). Following acclimation, flood water was discharged with a wet/dry vacuum, and microcosms were left saturated, but unflooded for 24 h, wherein composite plant and soil samples were collected from each block to determine initial [Se]. Figure 1 shows the equinox or solstice events and acclimation periods for each seasonal evaluation. Every two hours, internal water temperature and ambient air temperature was recorded with Watchdog Model 425 dataloggers (Spectrum Technologies, Inc., Aurora, IL).

Experimental design

The experiment was designed as a randomized complete block design nested within a split plot design. In each season, the main plot factor was weekly flood-discharge cycle (FDC) (n=4) defined as a six d flood period followed by a 24 h discharge period (Zhang and Moore, 1997). Subplot factor was a factorial arrangement of treatments where UNP, CAT, and DWD were treated with three levels of Se-impacted runoff consisting 0, 1x, and 2x Se rates (n=9). Subplot treatments were randomized within each weekly FDC block (Fig. 2B).

Selenium treatments

In the spring of 2018, fresh runoff was collected from a local mine to assess the water chemistry likely to occur from runoff over fly ash haul roads. Runoff was collected from a pond that retains runoff known to be impacted by SeO_4^{2-}. Because runoff was impacted by Se, a zero Se control solution was made with municipal water and adjusted to mimic the runoff water chemistry. Water chemistry results for across each weekly FDC are shown in Table 2. To meet the 1x (16 ppb) and 2x (32 ppb) Se target, runoff Se concentration was determined prior to making treatments and adjusted using Na_2SeO_4. Initial Se concentrations for 0, 1x, and 2x Se rates within each weekly are shown in Table 3.

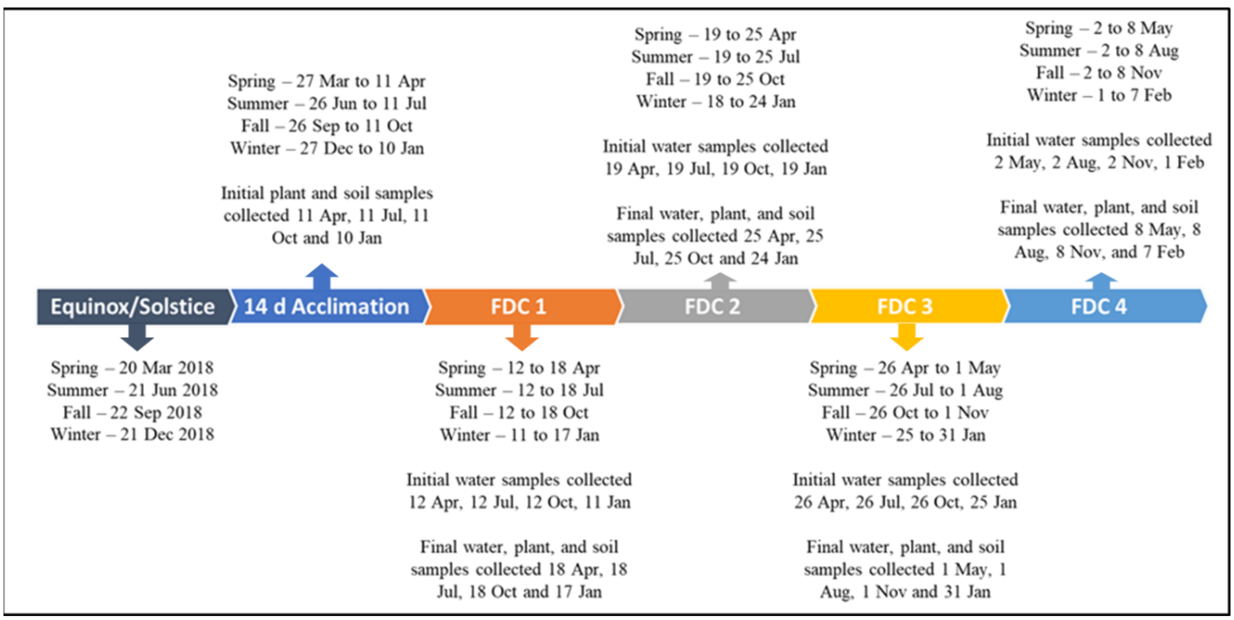

Figure 1. Dates for equinox and solstice events, 14-day acclimation period, and four weekly flood-discharge cycles (FDC) for the 2018-2019 seasonal microcosms study conducted at Mississippi State, MS. Water, plant, and soil sampling dates are included within each FDC.

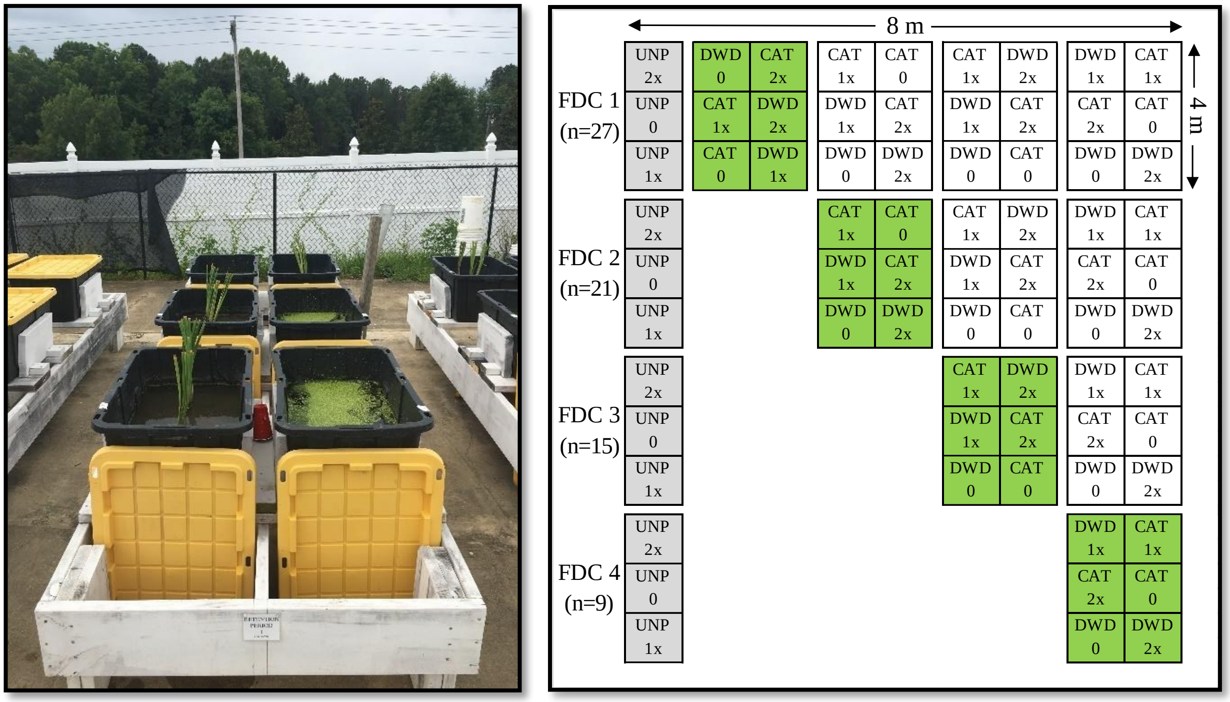

Figure 2. Picture (left) of cattail (CAT) and duckweed (DWD) planted microcosms within the first blocked flood-discharge cycle (FDC) and plot diagram (right) showing the experimental layout with CAT, DWD, and unplanted (UNP) microcosms crossed treated with one of three Se rates (0, 1x, or 2x Se).

Microcosms were flooded with 30 L of SeO_4^{2-}-impacted runoff and maintained in a flooded state for six d, followed by a discharge and subsequent 24 h period, wherein plant and soil samples were collected. This week-long flood-discharge-sample procedure was repeated across three weeks within each season.

In FDC one, 27 microcosms were flooded with 30 L runoff and evaluated for six d. At the end of FDC one, plant and soil samples were only collected from the gray and green highlighted blocks shown in Fig. 1. Only the planted microcosms, within FDC one (highlighted green), were destructively sampled and removed from the remainder of the evaluation. To minimize space and resources, UNP microcosms were carried forward for each FDC.

Table 2. Mean water chemistry results for Se treatments by season used in the microcosm study conducted at Mississippi State, MS from April 2018 to January 2019. (SD- standard deviation; SE- standard error (n=12))

Season	pH	EC	Hardness	Ca	Cl	K	Mg	Na	SO_4^{2-}
		dS/m	- - - - - - - -	- - - -	- - - -	ppm	- - - -	- - - -	- - - -
Spring									
Mean	7.03	1.68	609.3	212.0	148.5	84.8	23.6	66.2	623.2
SD	1.58	0.29	38.5	28.1	87.0	76.4	4.9	8.9	51.5
± SE	0.60	0.11	27.3	10.6	61.5	28.9	1.9	3.4	19.5
Summer									
Mean	8.87	1.95	509.9	182.8	341.5	16.6	12.9	227.4	464.8
SD	1.09	0.28	106.2	36.2	60.5	1.9	4.1	23.4	82.4
± SE	0.31	0.08	30.7	10.4	17.5	0.5	1.2	6.8	23.8
Fall									
Mean	8.63	2.08	573.8	196.6	353.8	21.6	20.2	237.5	534.7
SD	0.85	0.11	56.2	21.4	35.1	2.4	2.1	34.3	63.4
± SE	0.25	0.03	16.2	6.2	10.1	0.7	0.6	9.9	18.3
Winter									
Mean	7.81	2.25	592.7	205.0	362.7	16.4	19.6	245.7	589.0
SD	0.27	0.09	39.1	14.2	29.0	2.0	1.5	9.0	37.2
± SE	0.16	0.05	22.6	8.2	16.7	1.1	0.9	5.2	21.5

Table 3. Mean initial selenium concentration (ppb) (± standard error) for 0 Se, 1x Se, and 2x Se treatments in each flood-discharge cycle during the spring, summer, fall, and winter evaluations. For the 1x Se rate, the target Se concentration was 16 ppb. For the 2x Se rate, the target Se concentration was 32 ppb.

Season	Flood-discharge cycle 1 (n=9)	2 (n=7)	3 (n=5)	4 (n=3)	Season (n=24)
	- - - - - -	- - - - - -	ppb Se	- - - - - -	- - - - - -
Spring					
0 Se	0.6 ± 0.04	0.5 ± 0.04	0.7 ± 0.08	0.5 ± 0.10	0.6 ± 0.03
1x Se	16.0 ± 0.21	15.9 ± 0.29	16.7 ± 0.39	16.1 ± 1.16	16.1 ± 0.19
2x Se	32.7 ± 0.32	33.0 ± 0.63	36.2 ± 0.82	37.9 ± 1.69	34.1 ± 0.52
Summer					
0 Se	0.5 ± 0.04	0.5 ± 0.05	0.4 ± 0.06	0.4 ± 0.00	0.5 ± 0.02
1x Se	12.7 ± 0.22	12.7 ± 0.36	11.0 ± 0.23	6.2 ± 0.05	11.5 ± 0.46
2x Se	26.7 ± 0.26	23.6 ± 1.45	20.8 ± 0.55	11.8 ± 0.58	22.7 ± 1.06
Fall					
0 Se	0.6 ± 0.02	0.6 ± 0.00	0.6 ± 0.00	0.6 ± 0.02	0.6 ± 0.01
1x Se	18.3 ± 0.26	6.6 ± 0.27	16.2 ± 0.27	18.3 ± 0.27	14.4 ± 1.07
2x Se	35.0 ± 0.42	12.9 ± 0.23	30.7 ± 0.37	34.3 ± 0.19	27.6 ± 2.00
Winter					
0 Se	0.3 ± 0.03	0.2 ± 0.00	0.2 ± 0.00	0.5 ± 0.02	0.3 ± 0.02
1x Se	17.0 ± 0.27	17.8 ± 0.42	17.1 ± 0.17	16.8 ± 0.41	17.2 ± 0.18

2x Se	34.3 ± 0.60	36.4 ± 0.48	33.1 ± 0.31	34.3 ± 0.76	34.6 ± 0.37

Water sample collection

A 500-ml water sample was collected from all microcosms at treatment application and six d after application. To minimize dilution effects from pending precipitation events, microcosms were covered with yellow lids (Fig. 2) only for the duration of the precipitation event. Covering the microcosms also minimized the potential for cross contamination of Se treatments and potential transfer DWD into CAT microcosms. CAT plants were limber enough to be covered by lids. To account for sampling and evapotranspiration losses, water heights were recorded before and after sample collection. Prior to the final water sampling, microcosms were brought to volume based on the previously recorded height using municipal water. Municipal water [Se] was less than 0.33 ppb resulting in less than 2 µg Se mass added to the microcosm. Water samples were analyzed for total Se concentration [Se] with inductively coupled plasma mass spectroscopy (ICP-MS) (EPA Method 200.8) (Waypoint Analytical, Inc. Memphis, TN). Aqueous Se removal, as a percentage of applied Se, was calculated by the following equation:

$$\text{AqSeRemoval\%} = \frac{\text{Initial }[\text{Se}]_{\text{aq}} - \text{Final }[\text{Se}]_{\text{aq}}}{\text{Initial }[\text{Se}]_{\text{aq}}} \text{ X } 100 \qquad \text{Eq. 1}$$

Plant and soil sample collection

During the 24 h period following each flood-discharge cycle, plant and soil samples were only collected from microcosms within the designated sampling block. Individual CAT plants were harvested, rinsed free of soil and debris with a 1% (w:v) Alconox® solution (Alconox powdered precision cleaner, Alconox, Inc., White Plains, NY), rinsed with deionized water, and blotted dry with absorbent towels. DWD plants were removed from microcosms and rinsed clean with deionized water (Lo et al., 2015). Excess water was removed by applying gentle pressure on a fine-mesh sieve, then allowing samples to air-dry for 15 minutes. Total DWD biomass from each microcosm was separated into three subsamples. Individual CAT plants and DWD subsamples were weighed fresh, dried to a constant weight at 65°C, and re-weighed. Eight soil samples were collected from each microcosm and homogenized before analysis. Plant and soil samples were analyzed for total Se concentration by ICP-MS (EPA Method 6020B).

Statistical analysis

Within each seasonal evaluation, aqueous, plant, and soil [Se] were subject to analysis of variance (ANOVA). Significant main and interaction effects were determined by ANOVA (α=0.05) with PROC GLM. Where applicable, multiple comparisons were made using LS-means using a Tukey-Kramer adjustment.

Results and Discussion

Aqueous Selenium

Statistical Analysis. Water samples were analyzed for total Se concentration and data were analyzed for main and interaction effects. Seasonal data were analyzed separately with PROC GLM (α=0.05) and the results shown in Table 4. In each seasonal evaluation, significant main effects for flood-discharge cycle were observed. During the spring and fall evaluations, species main effects and interaction with FDC were observed. Selenium concentration was also significant in that greater Se removal was observed when Se increased from 0 to 1x or 2x.

Table 4. Analysis of variance (α=0.05) for aqueous selenium (Se) removal, as a percentage of applied Se, for the spring, summer, fall, and winter evaluations conducted at Mississippi State, MS.

Source	Spring	Summer	Fall	Winter
	- - - - - - - - - - Pr > *F* - - - - - - - - - -			
Flood-discharge cycle (FDC)	<0.001	<0.001	<0.001	0.002
Species (S)	<0.001	0.937	0.001	0.113
FDC X S	0.020	0.639	0.005	0.429
SeConcentration (Se)	<0.001	<0.001	<0.001	<0.001
S X Se	0.091	0.039	0.013	0.871

Temperature. For the duration of each evaluation, internal water and ambient air temperatures were recorded every two h (n=12). Minimum, maximum, and mean temperatures within each weekly flood-discharge cycle are shown in Table 5. Weekly mean temperatures were calculated by summing all recorded values divided by the number of recorded values. Seasonal mean temperatures were calculated by averaging weekly mean values. There were no differences between internal water and ambient air temperatures. Also, there were no differences among planted and UNP microcosms. Statistical analysis indicates a strong positive relationship between AqSeRemoval% and ambient air temperature (R^2=0.65, P <0.0001) (Fig. 3).

Table 5. Internal water and ambient air temperature (°C) during each flood-discharge cycle (FDC) for the seasonal evaluation conducted at Mississippi State, MS.

Season	Internal Water Temperature			Ambient Air Temperature		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Spring	- °C -					
FDC 1	0.6	28.9	14.3	0.6	28.9	14.3
FDC 2	5.7	32.4	17.3	4.0	43.5	17.2
FDC 3	6.1	37.9	20.9	4.9	37.9	20.1
FDC 4	12.9	43.5	23.8	12.5	44.9	23.3
Spring Mean	**6.3**	**35.7**	**19.1**	**5.5**	**38.8**	**18.7**
Summer						
FDC 1	20.6	40.6	31.0	20.6	46.4	29.4
FDC 2	22.1	39.7	29.7	22.1	50.7	29.7
FDC 3	19.5	40.8	28.8	21.7	42.0	29.0
FDC 4	21.1	42.5	30.5	22.5	44.0	29.7
Summer Mean	**20.8**	**40.9**	**30.0**	**21.7**	**45.8**	**29.5**
Fall						
FDC 1	10.9	29.9	18.5	7.7	42.0	18.8
FDC 2	5.7	32.4	14.3	1.9	29.1	13.1
FDC 3	9.8	26.8	18.1	7.7	34.9	17.7
FDC 4	7.3	26.0	14.6	5.3	41.6	14.8
Fall Mean	**8.4**	**28.8**	**16.4**	**5.7**	**36.9**	**16.1**
Winter						
FDC 1	0.6	20.6	7.2	-3.1	43.5	8.2
FDC 2	0.6	16.0	7.2	-4.1	39.7	8.3
FDC 3	-1.3	15.6	5.5	-5.1	45.4	7.9
FDC 4	0.1	28.3	15.2	-0.8	50.1	18.0
Winter Mean	**0.0**	**20.1**	**8.8**	**-3.3**	**44.7**	**10.6**

Selenium Removal. Overall, the greatest AqSeRemoval% was observed during the summer (73%) (P=<0.0001). AqSeRemoval% was similar during the spring (41%) and fall (42%) evaluations. During the winter evaluation, only 19% of the applied Se was removed. Results indicate greater AqSeRemoval% in microcosms planted with CAT (46%) and DWD (47%) compared with UNP (36%) microcosms (P=<0.0001).

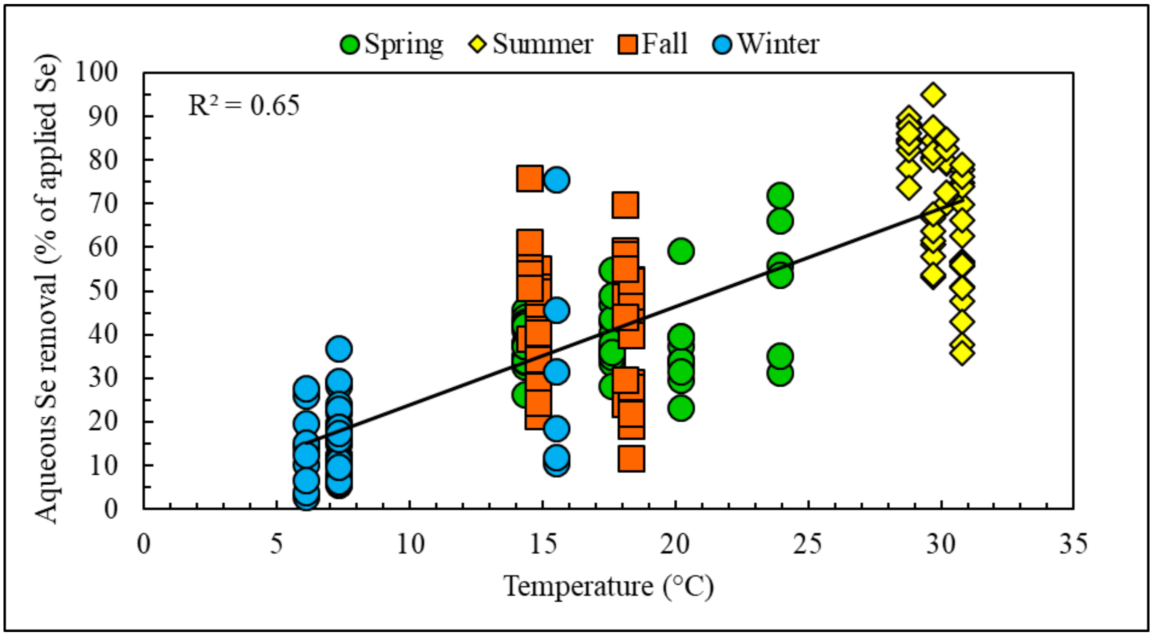

Figure 3. Relationship between aqueous Se removal and ambient air temperature.

Aqueous Se removal results for CAT, DWD, and UNP microcosms for the spring, summer, fall, and winter are shown in Fig. 4. During the third and fourth FDCs in spring (Fig. 4A), planted systems significantly increased AqSeRemoval% compared with UNP systems (P=0.0003). DWD-planted microcosms demonstrated greater Se removal compared with CAT (P=0.0001). Greater root surface area suspended in the water column would increase the rhizofiltration pathway compared to CAT. In CAT systems, Se would be adsorbed to the soil then taken up by the roots. In the summer (Fig. 4B), AqSeRemoval% was similar among planted and UNP microcosms throughout the third FDC. In FDC four, CAT and UNP demonstrated greater AqSeRemoval% compared with DWD. In CAT and UNP microcosms, volatilization is suspected as the primary elimination pathway. During the first and second FDCs of fall evaluation (Fig. 4C), CAT (47%) demonstrated the greatest AqSeRemoval% compared with DWD (24, 34%) and UNP (23, 26%) (P=0.0002). However, during the final two FDCs, planted microcosms demonstrated similar AqSeRemoval%. While we did not measure plant metabolism directly, CAT and DWD are reported to be photoperiodic (McNaughton, 1966; Oota, 1975). During the fall, CAT is likely increasing carbohydrate and nutrient reserves in response to decreasing photoperiod. Temperatures were 10 to 15 degrees lower compared to the summer evaluation (Table 5). These changes would likely impact microbial activity and may explain the AqSeRemoval% decrease in

UNP microcosms. Winter AqSeRemoval% was similar among planted and UNP system over the first three FDCs (Fig. 4D). However, in the fourth FDC of the winter, planted microcosms removed 41% of the applied Se compared with 15% in UNP microcosms. During this period, mean ambient air temperatures were increasing from 8°C to 18°C. Maximum ambient air temperatures were comparable to maximum air temperatures with similar Se efficiencies. Combined with extended day length, unusually high air temperatures would stimulate plant and microbial metabolic functions.

This study evaluated seasonal and plant species effects on aqueous Se removal in a detain-drain system supplied with SeO_4^{2-}. Results from this microcosm study support the hypothesis that aqueous Se removal varies with season and that CAT- and DWD-planted microcosms do enhance Se removal compared to UNP microcosms. Cattail and DWD are warm season plants that exhibit rapid growth and metabolic response to increasing temperature and photoperiod. Much of the research evaluating seasonal Se removal by CAT or DWD has been conducted in milder climates using sub-surface (Shardendu et al., 2003) or surface flow through microcosms (Allen, 1991; Zhang and Moore, 1997; Gao et al., 2003; Lin and Terry, 2003). We observed the greatest Se removal during the summer, followed by the spring and fall, and the least Se removal during the winter. Allen (1991) reported similar Se removal efficiencies and seasonal variability in CAT- and DWD-planted flow-through CWs. Gao et al. (2003) also found Se removal in CAT-planted flow-through to be greater in the summer compared to spring and fall.

Plant Selenium Accumulation

At the end of each weekly flood event, individual CAT plants and DWD biomass within each microcosm were collected and analyzed for total Se accumulation. Statistical analysis for main and interaction effects is shown in Table 6.

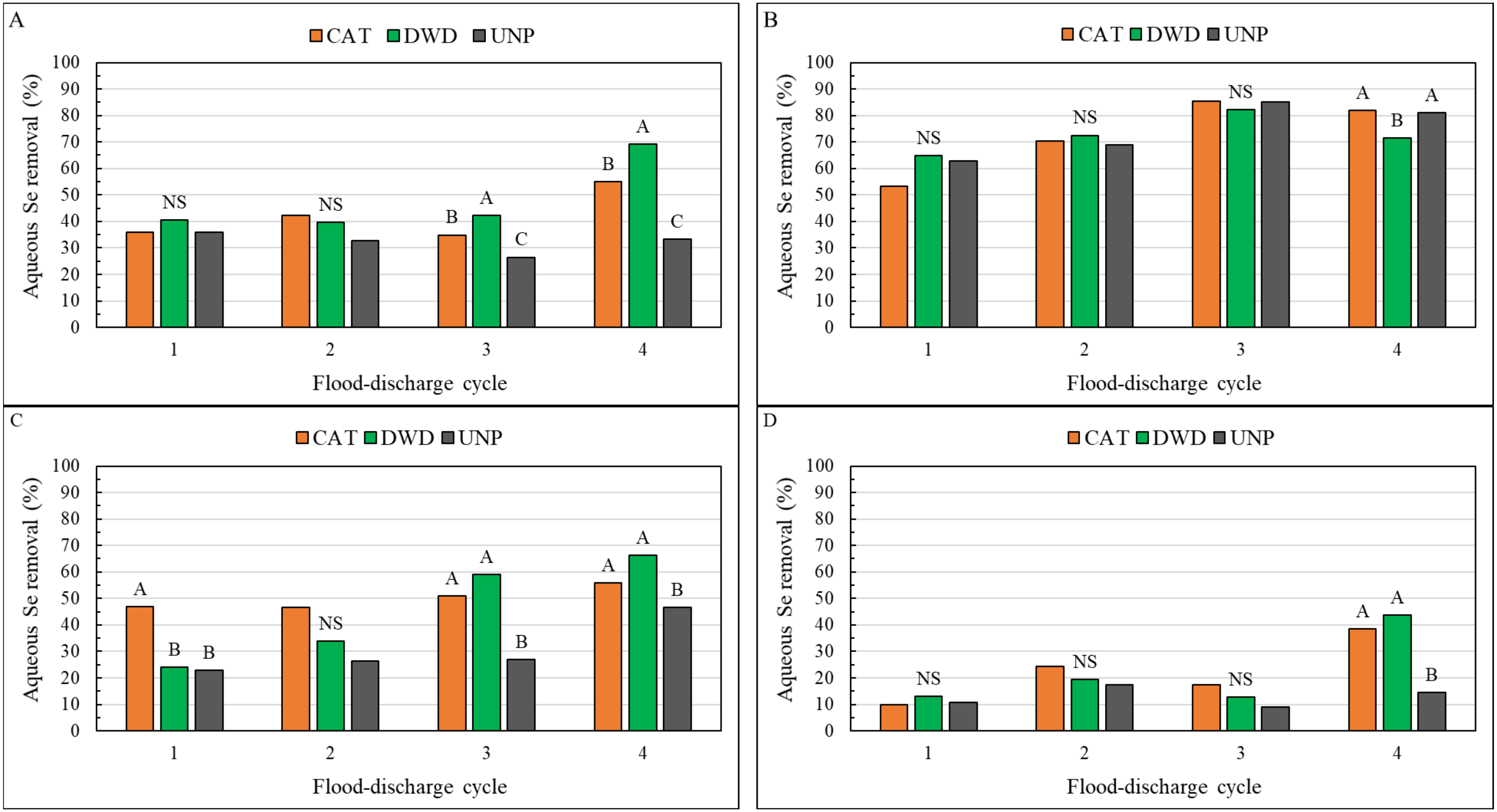

Figure 4. Mean aqueous Se removal, as a percentage of applied Se, in microcosms planted with cattail (CAT), duckweed (DWD), or unplanted (UNP) over four weekly flood-discharge cycles during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

Table 6. Analysis of variance (α=0.05) for plant selenium concentration for cattail and duckweed by season for the flood study conducted at Mississippi State, MS.

Source	Cattail				Duckweed			
	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
	Pr > F							
Flood-discharge Cycle (FDC)	0.8479	0.1466	<.0001	0.0609	<.0001	<.0001	<.0001	<.0001
SeConcentration (Se)	<.0001	<.0001	0.0359	0.1017	<.0001	<.0001	<.0001	<.0001
FDC x Se	0.3708	0.0334	0.0929	0.1296	<.0001	<.0001	<.0001	<.0001

Cattail. During each weekly FDC, CAT tissue [Se] was determined in microcosms flooded with simulated runoff with a control (0 Se), 1x, or 2x Se rates. Results for each FDC within each season are shown in Fig. 5. In zero Se control microcosms, tissue [Se] decreased linearly by 45 ppb during each weekly flooding event in the spring (R^2=0.88), summer (R^2=0.53), fall (R^2=0.82). In each season, greater tissue [Se] was observed in Se-treated microcosms compared to control microcosms and generally increased with each successive flood event. During the spring (Fig. 5A) and the summer (Fig. 5B), tissue [Se] generally increased with increasing aqueous [Se] but did not exceed 700 ppb Se in either season. During the final FDC, Se-treated microcosms, plant population increased from three to six. Carvalho and Martin (2001) also noted fresh biomass increased when exposed to elevated Se levels. Increased biomass may be a plant defense mechanism to dilute tissue [Se]. In the fall (Fig. 5C) and winter (Fig. 5D), tissue [Se] varies among FDCs. CAT is a warm-season plant and responds positively to long day photoperiod. Although CAT plants were transplanted from the same ramet and visually blocked according to size, variability among plants is expected. Overall, tissue [Se] and soil [Se] did not increase enough to account for the decrease in aqueous [Se]. These results suggest the potential for CAT-planted systems to phytovolatilize Se.

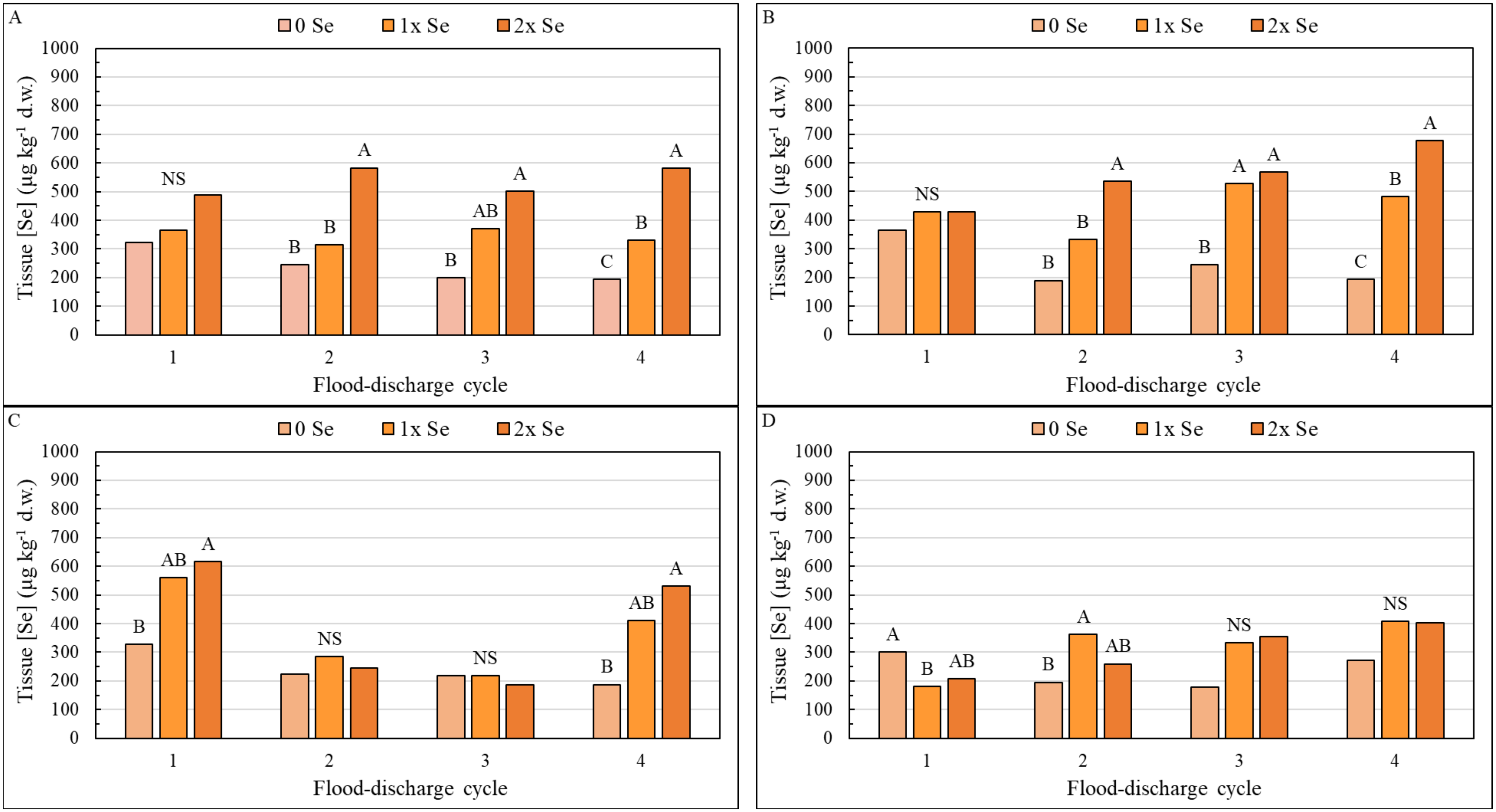

Figure 5. Mean cattail plant tissue selenium concentration ([Se]) over four weekly flood-discharge cycles flooded with 0 Se, 1x (16 ppb), or 2x (32 ppb) Se rates during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

Duckweed. Mean DWD tissue [Se] was an order of magnitude greater compared with CAT (Fig. 5). In general, tissue [Se] increased with increasing aqueous [Se] as a linear or polynomial function of successive Se applications. In the spring (Fig. 6A), Se uptake rates are similar between microcosms treated with 1x or 2x Se rates, but tissue [Se] does increased with successive Se application. In the summer (Fig. 6B), tissue [Se] increases about 2000 μg kg^{-1} with each Se application until the fourth FDC. During the fourth FDC, decreased tissue [Se] may be the result of a dilution effect from decreased Se application. Initial aqueous [Se] were only 6 ppb (1x Se) and 12 ppb (2x Se rate) (Table 3). In the fall (Fig. 6C), tissue [Se] generally increases until the final FDC. This may be explained by decreasing temperature and photoperiod. In the winter (Fig. 6D), decreased Se uptake corresponds to decreased aqueous Se removal (Fig. 4D). One anomaly is the large tissue [Se] increase in the 2x Se treated microcosms during the final FDC. This may be explained by the decrease in plant dry weight, which would concentrate the Se in tissues. This increase in tissue [Se] is consistent with AqSeRemoval% for this FDC (Fig. 4D).

Rhizofiltration is a primary Se elimination pathway in that suspended roots of DWD have a greater surface area that increases Se absorption. DWD is a known Se hyperaccumulator (Allen, 1991; Zayed et al., 1998; Carvalho and Martin, 2001; Miranda et al., 2014; Lo et al., 2015). Increased fresh biomass and visual appearance of enhanced plant health were observed in microcosms treated with the 2x Se rate compared to the zero Se control and 1x Se rate. Miranda et al. (2014) also observed a 1.5-fold increase in DWD fresh biomass when supplied with Se.

Increasing temperature associated with increasing photoperiod drive plant growth this linear trend. [Weekly mean temperatures water and air temperatures ranged from 20°C (min) to 50°C (max) are between 14 and 25°C (Table 5).] However, during the summer, water and air temperatures ranged from 20°C (min) to 50°C (max). Elevated temperatures increase plant metabolic processes, such as transpiration and respiration, requiring more water, thus greater aqueous [Se] would increase tissue absorption (Allen et al., 2002). Also, greater Se uptake in the summer compared to the spring and fall could be from a Se reduction from SeO_4^{2-} to SeO_3^{2-}. A surface covering of DWD would limit O_2 exchange with water surface air. Dissolved O_2 concentrations are temperature dependent in that elevated temperatures decrease O_2 concentrations, thus reducing the system.

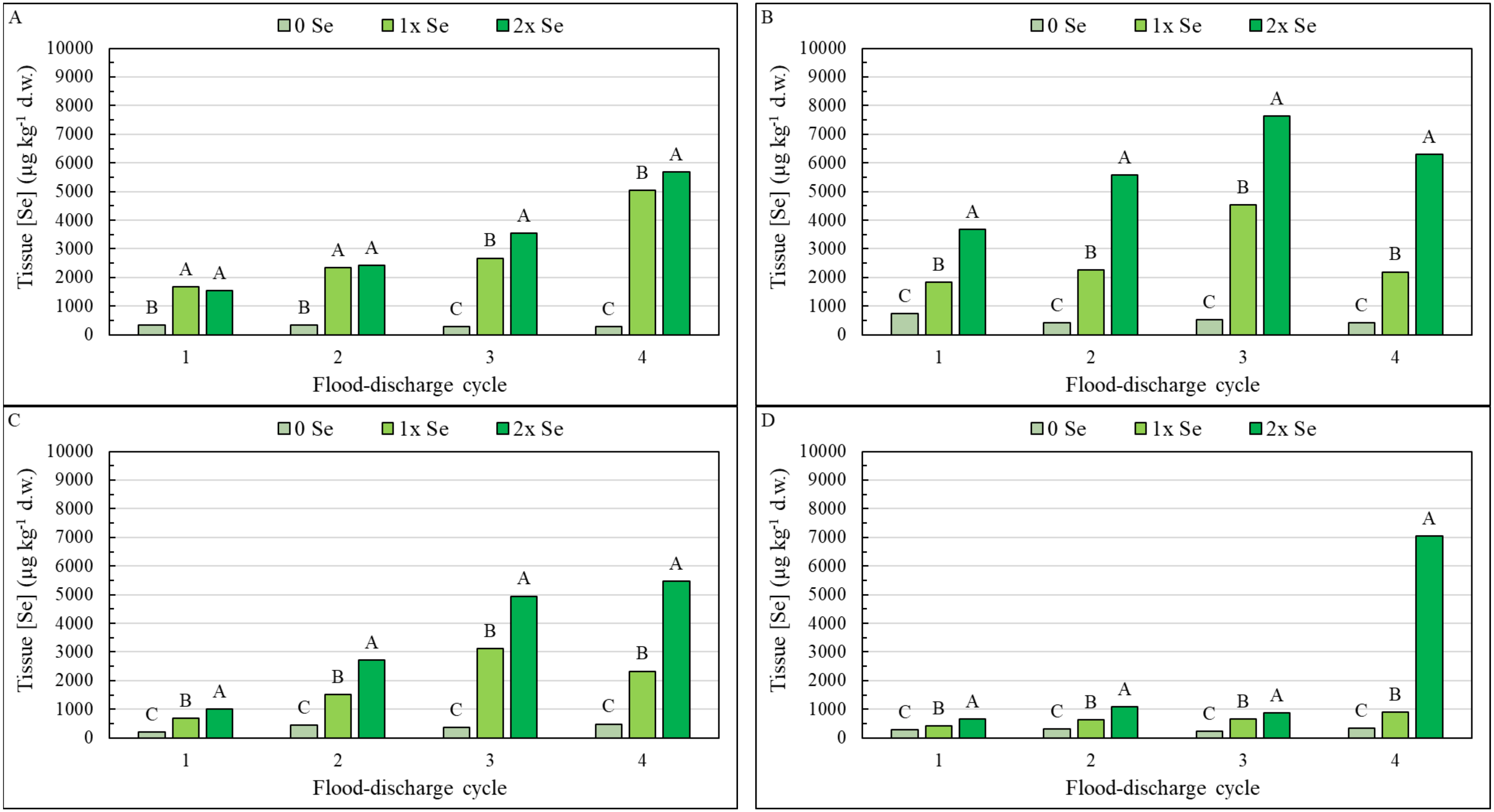

Figure 6. Mean duckweed plant tissue selenium concentration ([Se]) over four weekly flood-discharge cycles flooded with 0 Se, 1x (16 ppb), or 2x (32 ppb) Se rates during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

Soil Selenium

Baseline soil samples were collected prior to Se application and at the end of each weekly FDC. Statistical analysis is shown in Table 7. Initial and final soil [Se] for CAT (Fig. 7), DWD (Fig. 8), and UNP (Fig. 9) over four weekly flood-discharge cycles for each seasonal evaluation are shown below. In all seasons, weekly FDC was the only significant effect observed.

Table 7. Analysis of variance (α=0.05) for soil selenium concentration for the spring, summer, fall, and winter evaluations conducted at Mississippi State, MS.

Source	Spring	Summer	Fall	Winter
	- - - - - - - - - - Pr > *F* - - - - - - - - - -			
Flood-discharge cycle (FDC)	0.0100	<.0001	<.0001	<.0001
Species (S)	0.7721	0.4650	0.3765	0.3850
FDC X S	0.8598	0.0752	0.4750	0.6286
Se Concentration (Se)	0.8592	0.0027	0.0829	0.0816
S X Se	0.6472	0.2640	0.4696	0.1108

Results from this study indicate little increase in soil [Se]. One explanation for this is soil Se volatilized during the 24 h discharge period. It is possible that elimination pathways vary between flow-through and detain-drain designs. For example, in a surface flow-through CW, Gao et al. (2003) reported 36% of the applied Se was adsorbed by the soil. Under continuously flooded conditions, Se would likely be reduced and immobilized in the soil (Hansen et al., 1998). Zhang and Moore (1997) reported greater Se volatilization when microcosms were flooded and drained compared to continuously flooded. Selenium volatilization is a complex elimination pathway involving dynamic interactions among Se chemical form, temperature, air flow, flooding-drying cycles, and microbial populations (Doran and Alexander, 1977; Karlson and Frankenberger, Jr., 1988; Frankenberger, Jr. and Karlson, 1989; Thompson-Eagle and Frankenberger, Jr., 1990; Azaizeh et al., 1997; Zhang and Moore, 1997; Lin et al., 1999).

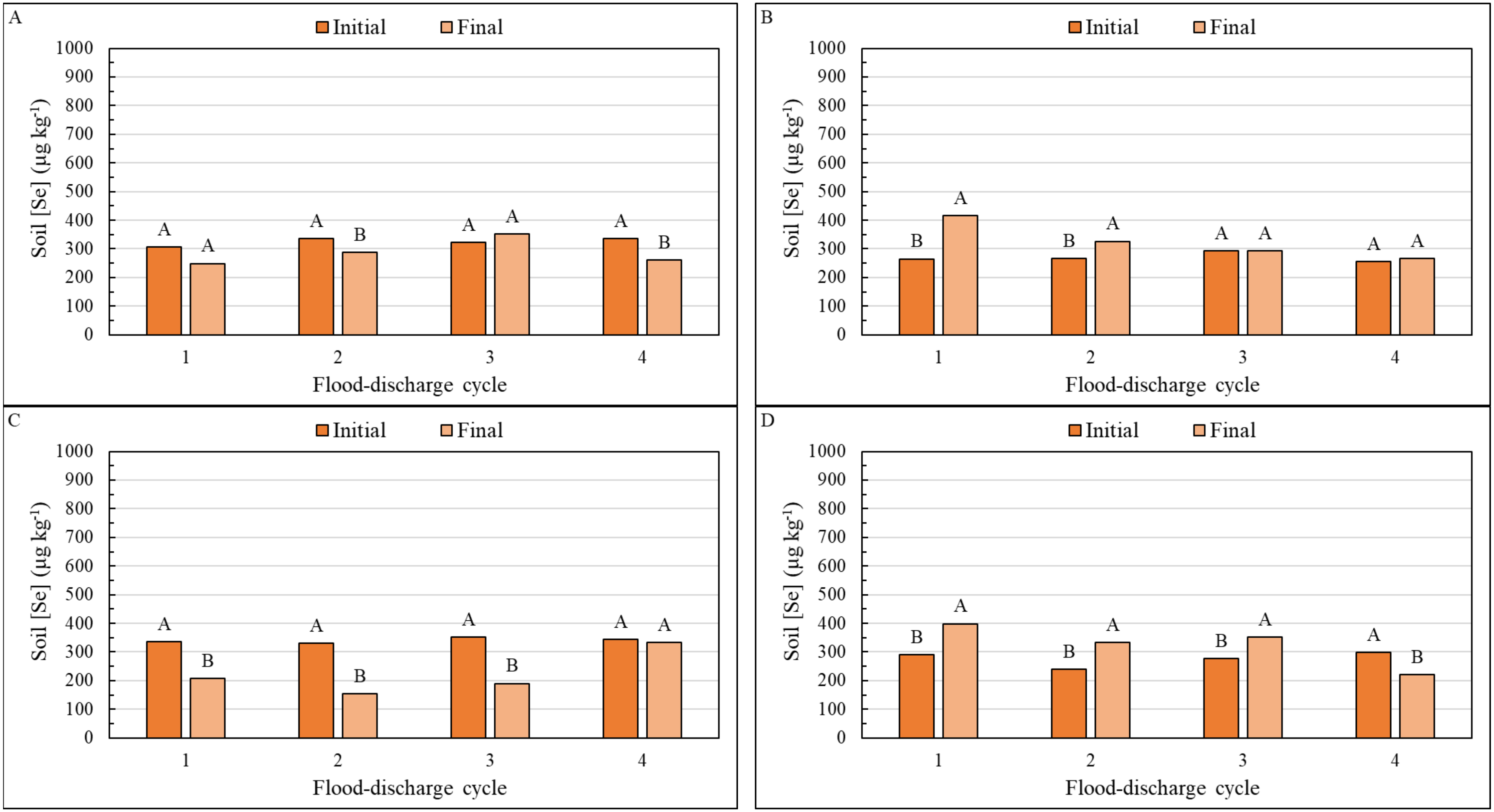

Figure 7. Initial and final soil selenium concentration [Se] over four weekly flood-discharge cycles in cattail (CAT) planted microcosms during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

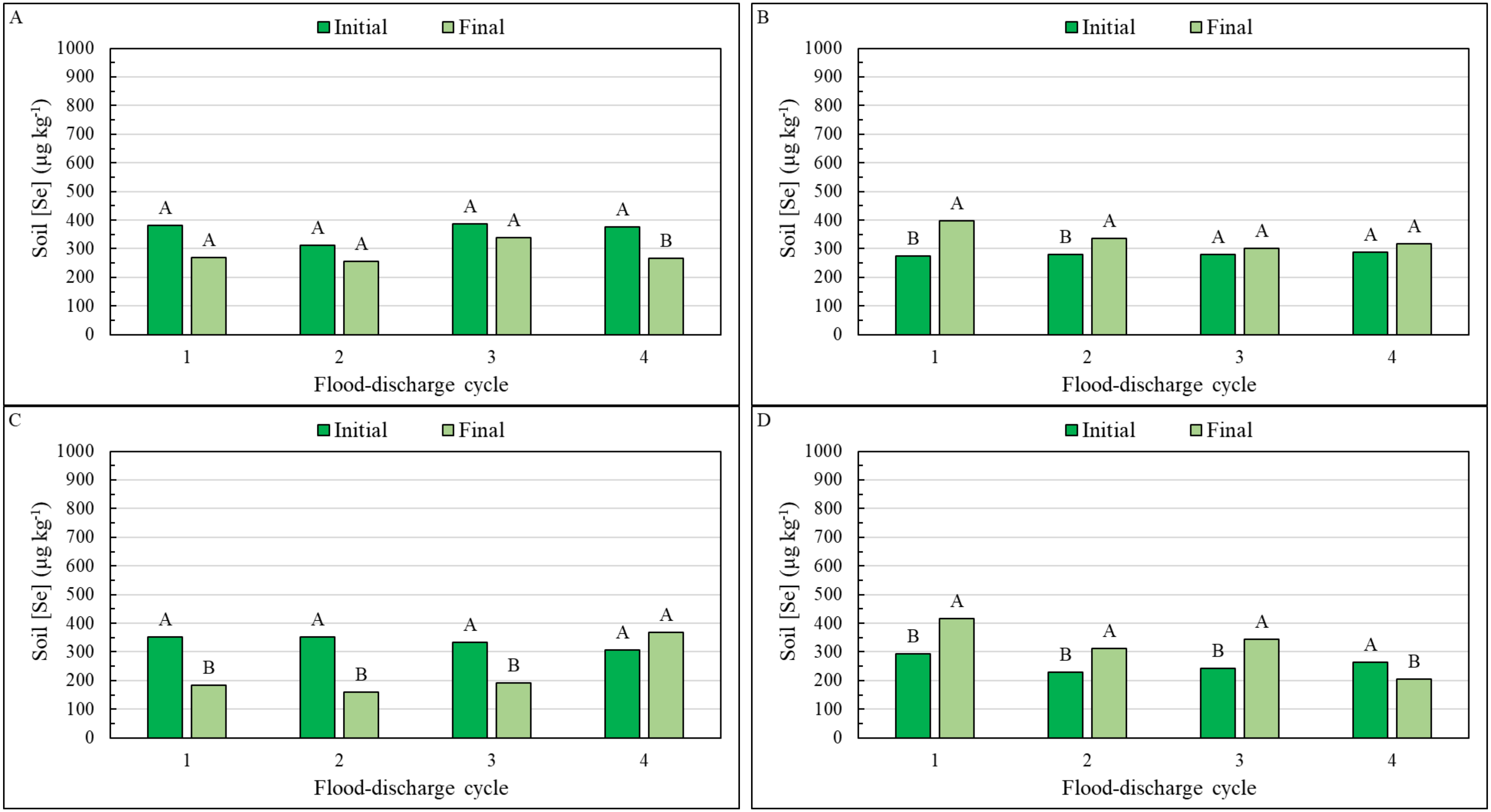

Figure 8. Initial and final soil selenium concentration [Se] over four weekly flood-discharge cycles in duckweed (DWD) planted microcosms during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

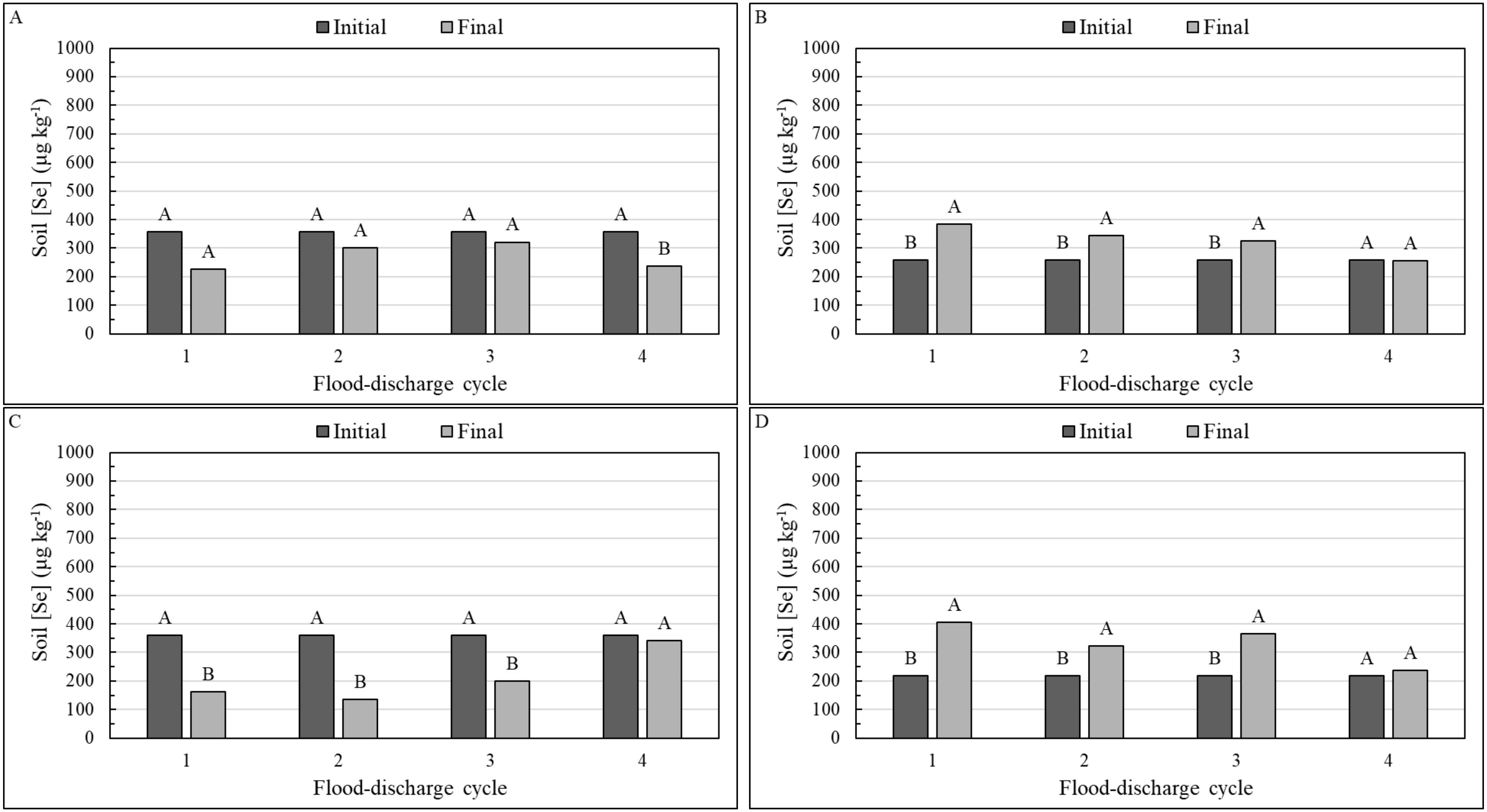

Figure 9. Initial and final soil selenium concentration [Se] over four weekly flood-discharge cycles in unplanted (UNP) microcosms during the spring (A), summer (B), fall (C), and winter (D) evaluations conducted at Mississippi State, MS. Treatment means with same letter are not statistically different (α=0.05).

Conclusions

Results from this study indicate season influences AqSeRemoval% in planted and UNP microcosms. When ambient temperatures range between 15 and 20°C, CAT- and DWD-planted microcosms significantly improve water quality of SeO_4^{2-}-impacted runoff. Both species are well adapted to many regions of the US and should be considered when selecting aquatic species for CW phytoremediation. In regions with shorter growing seasons or colder temperatures, covering small-scale CWs with a greenhouse or high-tunnel may increase ambient air temperatures and extend Se removal periods. Research evaluating cold-tolerant aquatic species for Se removal should be considered to provide year-round Se removal.

In CAT and DWD, rhizofiltration is an important elimination pathway for decreasing aqueous [Se], but subsequent metabolism differs between species. Although CAT tissue Se concentration did increase, we suspect CAT to subsequently phytovolatilize Se from the system. Lin (2008) observed high Se volatilization rates during late spring and summer months with high temperature. Zhang and Moore (1997) reported soil Se volatilization was 2x greater at 30°C compared to 20°C, and 8x greater compared to 10°C. On the other hand, DWD clearly accumulates Se. This can be problematic. Duckweed is a food source for fish and other wildlife and excessive tissue Se concentration increases the potential for deleterious effects through biomagnification. CWs planted with DWD may need to be harvested and replaced with fresh DWD. Fortunately, there is potential for harvested DWD to provide a secondary income as a feedstock for biofuel or used for biofortification in human and livestock diets that are Se deficient.

Finally, CW phytoremediation does appear to be an effective management strategy for ameliorating SeO_4^{2-}-impacted runoff. However, dynamic interactions between abiotic and biotic factors confound accurately determining the fate and distribution of Se in open-environment experiments. A follow-up experiment is being conducted to better describe Se elimination pathways and determine a Se mass balance in CAT, DWD, and UNP microcosms.

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