Nitrogen uptake potential of two wetland plants under varied water temperatures and nitrogen concentrations when grown as floating treatment wetland

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Research Article

Keywords: Irrigation return flow, Nitrate remediation, Helianthus maximiliani, Asclepias speciosa, Runoff water quality, Retention pond, Bioremediation

Posted Date: July 2nd, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4124953/v1

Abstract

Excessive use of commercial fertilizers in nursery production results in substantial fertilizer runoff, leading to surface and groundwater contamination. Therefore, to prevent contamination and comply with regulations, irrigation return flow (runoff water) is generally collected in retention pond before it can be safely discarded or released. Nitrogen, applied as nitrate compounds is among the highest used fertilizer and is prone to leaching and runoff. Employing a phytoremediation strategy to remediate irrigation return flow (runoff water) has the potential to lower nitrate concentrations in water before release. In this study, we assessed the suitability of growing *Helianthus maximiliani* (maximilian sunflower) and *Asclepias speciosa* (showy milkweed) in a floating treatment wetland system (FTW) and evaluated the nitrate uptake capacity of these plants grown in three different nitrogen concentrations i.e., 10, 20, and 30 mg/L and two water temperatures of 75°F and 85°F. Increasing water temperature increased growth index for both species and increased dry weight for *Asclepias speciosa*. However, for *Helianthus maximiliani*, increasing temperature had a lesser t effect on total dry weight. The accumulation of nitrate nitrogen in plant tissue was higher in *Helianthus maximiliani* at the nitrate nitrogen concentration of 20 mg/L under both temperature treatments. However, in *Asclepias speciosa*, the accumulation of nitrate nitrogen in plant tissue was higher at the high temperature treatment of 85°F and 20 mg/L nitrate nitrogen concentration. The nitrate nitrogen uptake from both plants in this study indicated suggests that *Helianthus maximiliani* ‘maximilian sunflower’ and *Asclepias speciosa* ‘showy milkweed’ can be grown as FTW and be used to remove nitrogen from a nursery retention pond.

Introduction

As per 2022 National Water Quality Assessment study, agricultural activities are among the major sources of surface water pollution in U.S. It is the leading cause of impairment to rivers and streams, the second leading source of pollution for wetlands, and the third leading source of pollution to lakes (US EPA 2015). Excessive use of commercial fertilizers in agricultural production systems results in substantial fertilizer runoff, making fertilizers the primary pollutant originating from agricultural practices (Chen et al. 2018; Jabbar & Grote, 2019; Kourgialas et al. 2017). Among fertilizers, nitrogen (N) and phosphorus (P) are major plant nutrients that can limit plant growth therefore, they are applied often applied excessively to ensure optimal plant growth and performance. However, this excessive application frequently results in runoff, leading to water pollution. (Mouri et al. 2011; Yu et al. 2015). Typically, only 2–10% of applied nitrogen accumulates in crops, while the remaining portion can leach into water, potentially affecting the health of plants, animals, and humans (Fields 2004; Sengupta et al. 2015). Nitrogen runoff contributes to eutrophication, leading to depleted oxygen levels in water, which can be fatal to aquatic life. Additionally, nitrogen runoff causes harmful aquatic algal blooms, which produce neurotoxins capable of affecting both animals and humans (Baron et al. 2013; Chislock et al. 2013; US EPA 2013). Leaching a substantial amount of N into underground aquifers results in groundwater pollution. These underground aquifers vital drinking water source in the U.S., hence such pollution has the potential to severely restrict access to clean drinking water (Foley et al. 2012). Harmful algal blooms are present across all 50 states of the United States, with notable occurrences concentrated in the Chesapeake Bay, Lake Erie and the Gulf of Mexico. Therefore, in an effort to protect both surface and groundwater from nitrate (NO_3^-) pollution, the U.S. Environmental Protection Agency (EPA) has imposed regulations limiting the discharge of water containing nitrate-nitrogen (NO_3–N) concentrations exceeding 10 mg/L or 45 mg/L when expressed as (NO_3^-) (U.S. EPA 1986).

There are various ways to remediate NO_3^- laden water resulting from N-related runoff (Ali et al. 2013; Kafle et al. 2022). One easy and low-cost way to remediate NO_3^- is through bioretention. Bioretention is a pollution remediation technique that uses soil, plants, and microbes to clean contaminated water before it discharges. This technique is frequently utilized to eliminate NO_3^- from water. Bioretention involves multiple degradation processes like adsorption, ion exchange, photodegradation, and microbial transformation, making it effective for removing nitrates from water. In a study conducted by Li et al. (2021), it was demonstrated that employing a bioretention system effectively removed 72.9–100% of NO_3–N from wetlands. Constructed wetlands are modified bioretention systems that extensively employ plants to remove contaminants and are equally or even more effective at removing NO_3^- than traditional wastewater treatment methods. The removal of contaminants from water using wetland plants is called phytoremediation (Fox et al. 2008; Ojoawo et al. 2015; Su et al. 2019). The use of a constructed wetland to remove N from irrigation return flow generated from a commercial nursery indicated that NO_3–N remediation efficiency averaged 94.7% during spring and fall and 70.7% during winter (Taylor et al. 2006). Research by McMaine et al. (2020) demonstrated a NO_3^- removal rate exceeding 78% from irrigation return flow generated from nurseries and greenhouses using constructed wetlands.

A study by Henderson et al. (2007) concluded that vegetated mesocosms, artificial setups resembling natural wetlands in water dynamics and ecological functions but designed for research purposes, removed 63 to77% more N from constructed wetlands than non-vegetated mesocosms. The efficacy of phytoremediation, among many other factors, also depends on plant type, target contaminant, and concentration of the contaminant in water (Kirk & Kronzucker 2005). In a study by Jamshidi et al. (2014), the wetland plants *Phragmites* sp. and *Typha* sp., removed 79% and 77% of N and 21% and 14% of P, respectively. Wetland plants such as *Typha latifolia, Juncus effusus* (soft rush), *Iris pseudacorus* (common reed), and *Phragmites australis* are commonly employed for N removal in wetlands. Several other wetland species such as *Sagittaria lancifolia* (bultongue arrowhead), *Pontederia cordata* (pickerelweed), *Canna × generalis* 'australia', *Scripus Validus* (bulrush), and *Cyperus haspan* (dwarf papyrus) have demonstrated effectiveness in N removal (Chen et al. 2009; Read et al. 2008; Srivastava et al. 2008; Stearman et al. 2003). In a study by Wang et al. (2002), wetland plants *Polygonum amphibium* (sharp dock) and *Eichhornia crassipes* (water hyacinth) had N uptake of 63.6 and 42.8 mg of NO_3–N per kg plant dry weight, respectively. For *Lemma minor* (duckweed), *Oenathe javanica* (water dropwort), and *Lepironia articulata* (calamus), N uptake ranged from 20–30 g per kg of plant dry weight. In a study by Kirumba et al. (2015), *Pontederia cordata* (pickerelweed) reduced water NO_3^- concentration in wetlands by 50–70%. Thus, a wide range of wetland plants have the potential to remove NO_3^- from runoff water with various efficacy rates. Floating treatment wetlands (FTW) function similarly to constructed wetlands yet they differ in their permanence (White 2021). While constructed wetlands are typically permanent to semi-permanent structures, FTW systems offer flexibility. They can be effortlessly installed and removed at various sites and across different time frames. Additionally, in FTW, entire plants can be easily harvested, facilitating the relocation of biomass between locations to optimize efficiency. This convenient plant removal feature of FTW also helps prevent the release of nutrients back into the water if entire biomass are removed (Chen et al. 2009).

Even after 50 years of the Clean Water Act in U.S., almost half of rivers and streams in U.S. are impaired. In Utah, 152,691 acres of lakes and ponds are classified as impaired (Larsen 2022). Nitrogen is one of the key sources of water impairment (Manuel 2014) and phytoremediation employing FTW is one of the efficient way to remove N from water. *Helianthus maximiliani* (maximilian sunflower) and *Asclepias speciosa* (showy milkweed) are common wetland plants grown throughout the United States, including Utah. These plants are non-invasive, aesthetically attractive, and thrive in wetlands. However, their applicability for FTW and their efficiency in removing nitrates is still unknown.

Nutrient absorption by plants depends on root temperature and, for FTW that is water temperature (Reddy & De Busk, 1985), as root zone temperature affects photosynthesis and growth. In a study by Ali et al. (1996), NO_3^- transport from roots to leaves decreased as root temperature was reduced from 68℉ to 54℉ F. Similarly, increase in root temperature using TGRooZ, a device that generates a temperature gradient, showed positive effect on shoot growth (González-García et al., 2022). Therefore, by controlling and maintaining root zone temperature in FTW, it may be possible to enhance plant growth and nitrate uptake. Therefore, the objective of our research was to 1) to assess the suitability of growing *Helianthus maximiliani* and *Asclepias speciosa* in a FTW system, 2) to evaluate the NO_3^- uptake capacity of these plants under three distinct N water concentrations, and 3) to determine the impact of water temperature on NO_3^- uptake.

Materials and methods

Plant materials and growth conditions

This study was conducted at the Utah State University (USU) Research Greenhouse in Logan, UT, USA, for three months. Wetland plants, *Helianthus maximiliani* (maximilian sunflower) were purchased from Aquatic and Wetland Nursery, LLC (Lupton, CO, USA) and *Asclepias speciosa* (showy milkweed), was purchased from North Fork Native plants (Rexburg, ID, USA). The plants were acclimatized in the greenhouse in 4-inch plug trays for two weeks before transplanting into the FTW systems. Acclimatization was accomplished by submerging the plug trays in potable water [EC = 0.35 ± 0.01 $dS \cdot m^{-1}$; pH = 7.7 ± 0.2], which was refilled as needed.

After two weeks, plants were transferred into an HDX 12-gallon storage tank with a lid perforated with six, 2-inch holes (Fig. 1, left). There were 12 tanks in total and each tank held six replications of one plant species at specific temperature and N levels (Fig. 1, right). Each tank had a volume of 0.073 m^3 with the capacity to hold 45L of water. Two-inch neoprene plant collars and net cup pots were used to hold the plants in each perforated hole of the storage tank lids. The plants were grown for three months in the greenhouse. The temperature inside the greenhouse remained within the range of 70° and 75° F throughout the entire research period. Water temperatures were maintained at the desired levels using heating pads placed beneath the storage tanks.

Experimental setup and design

Treatments included two water temperatures of 75°Fand 85°F and three NO_3–N concentrations of 10, 20, and 30 mg/L. BN-LINK heat mats (BN-LINK INC. El monte, CA, USA) were placed below the storage tanks along with digital thermostat controllers in the water to ensure a constant temperature of either 75° or 85° F. RC-5 temperature data loggers (Elitetech, San Jose, CA, USA) were kept above the heat mats to record mat temperatures (Fig. 2). The twelve storage tanks were placed in two rows, with six tanks allocated to each temperature level (75° and 85° F). Each treatment combination had six replications of single each plant species. An air pump (VIVOSUN, Ontario, CA, USA) continuously circulated air within each tank to ensure proper root aeration throughout the research period.

Water sampling, analysis, and calculations

The tanks were filled with 33L of water, each containing NO_3–N concentrations of either 10, 20, or 30 mg/L. Except for N, all other nutrients were applied at the rates of 10 mg/L of P as P_2O_5, 30 mg/L of K as K_2O, and 4 ml/L of micronutrients per tank per L of water (Pendleton turf supply, Waterford, WI, USA) in each tank. The nutrient solution in each tank was refilled every 8 to 10 days with the same concentration of fertilizers to maintain 32 L of water at the desired nutrient concentrations. The chemicals used to supply the desired quantities of nutrients were potassium monophosphate monobasic (KH_2PO_4; Sigma-Aldrich, St. Louis, MO, USA), potassium nitrate (KNO_3; Sigma-Aldrich, St. Louis, MO, USA) and calcium nitrate ($Ca(NO_3)_2$) (Fisher scientific, Fair Lawn, NJ, USA). The chemicals were precisely measured to achieve the desired N and other nutrient concentrations and manually mixed in the tanks after addition to maintain homogeneity. Nitrogen concentrations in the tanks were analyzed using an HI83306 Environmental Analysis Photometer (Hanna Instruments Inc., Woonsocket, RI, USA) to confirm the desired concentrations before the start of the experiment (Table 1).

Table 1
Actual vs. nominal concentrations of nitrate-nitrogen from fertilizer

Nominal NO_3^--N concentration (mg/L)	Actual NO_3^--N concentration (mg/L)
10	8.3 ± 0.81
20	15.7 ± 1.35
30	26.3 ± 2.40

Water samples were collected from each tank 8 weeks after the treatment was initiated. All samples were taken six days after the last refill of nutrients in the tanks between 10:00 and 11:00 a.m., ensuring adequate time for uniform distribution of nutrients within the tank. Each water sample was then analyzed for NO_3- using the cadmium reduction method employed by the HI83306 Environmental Analysis Photometer (Hanna Instruments Inc., Woonsocket, RI, USA). We also calculated the total NO_3-N applied to each tank, the remaining NO_3-N in the tank, and the total NO_3-N lost from the tank using following equations:

Total Nitrate Nitrogen applied (mg) = Total water applied (L) × NO_3-N concentration (mg/L).

Remaining Nitrate Nitrogen in tank (mg) = Remaining water in tank(L) × Tank NO_3-N concentration (mg/L).

Total Nitrogen loss from tank (mg) = Total NO_3-N applied (mg) - Total NO_3-N remaining (mg)

Plant sampling and analysis

Three replications of both species were harvested before the experimental treatments were applied, oven-dried at 60°C for three days and sent to a commercial plant laboratory (Water Agricultural Laboratories, Camilla, GA, USA) to determine the initial tissue N concentration of both species (Table 1).

After the experiment was completed, the roots and shoots of both species were sampled and weighed to determine fresh root and shoot weights. They were then dried at 60°C for three days to determine dry weights. The root and shoot materials were mixed and sent to a commercial plant laboratory (Water Agricultural Laboratories, Camilla, GA, USA) to determine whole plant N concentrations. The NO_3^- ion selective electrode (ISE) method was employed to conduct tissue nitrogen analyses (Cunniff and Association of Official Analytical Chemists International, 1995). Total NO_3-N per kg of dry weight was calculated in the laboratory, however, we also calculated total NO_3-N accumulation per plant using the following formula:

Total NO_3-N accumulation per plant (mg) = tissue NO_3-N concentration (mg/kg) × average plant dry weight (kg).

Table 2
Initial tissue nitrogen concentrations in *Asclepias speciosa* (showy milkweed) and *Helianthus maximiliani* (maximilian sunflower)

Wetland plants	**Initial tissue nitrate-nitrogen concentration (mg/kg of dry weight)**
Asclepias speciosa (showy milkweed)	1.42 ± 0.17
Helianthus maximiliani (maximilian sunflower)	9.25 ± 4.94

Plant growth and physiology

The length of all the stems of each plant was measured from the base of the stem and averaged to calculate the stem length for each replication. This average stem length was used as a proxy for plant growth. The average stem length was measured at the end of the second month after treatment application. The roots of each plant were cleaned and arranged to measure the root length using measuring tape. We also measured total chlorophyll content (CPHLT), Chlorophyll Content Index (CCI), Greenness index (G), Soil Plant Analysis Development (SPAD) and Normalized Difference Vegetation Index (NDVI) using a CI-710s Spectra Vue Leaf Spectrometer (CID Bio Science, Inc., Camas, WA, USA) before destructive harvesting.

Statistical Analysis

The data was analyzed with Minitab statistical software 21.3.1 (PA, USA) using a two-factor factorial general linear model where temperature and NO_3-N concentration were the two factors we also did the regression analysis to observe particular trends in data. Data were checked for uniformity and homogeneity of variances before conducting statistical analyses. Post-hoc testing was conducted using the Tukey method ($p < 0.05$). The data are expressed as mean ± standard error.

Results

Average plant growth in response to temperature and nitrogen concentration

Increasing N concentration did not change plant growth as determined by average stem length of *Asclepias speciosa* at the higher temperature but did increase plant growth at the lower temperature ($p = 0.04$) (Fig. 3). For *Helianthus maximiliani*, neither N concentration ($p = 0.261$) nor N × temperature interaction ($p = 0.201$) affected average stem length. However, increasing temperature did increase plant growth (Fig. 4).

Total dry weight in response to temperature and nitrogen concentration

Increasing the water temperature from 75°F to 85°F increased the total dry weight ($p = 0.00$) of *Asclepias speciosa* but did not change the total dry weight ($p = 0.51$) of *Helianthus maximiliani*. In addition, the interactive effect of temperature and nitrogen concentration was only observed in *Asclepias speciosa* for total dry weight and root dry weight but had no effect on total dry weight and root dry weight of *Helianthus maximiliani*. However, the interaction of the low water temperature of 75°F and 30 mg/L N concentration on *Asclepias speciosa* plants resulted in similar dry weights to 85°F and any N concentration (10, 20, and 30 mg/L). Decreasing N concentration below 30 mg/L at 75°F water temperature in *Asclepias speciosa* decreased total dry weight (Fig. 5, C). Shoot and root dry weight increased at 85° F, and root dry weight decreased at 75° F below 30 mg/L N in *Asclepias speciosa* (Fig. 5, A and B). Root dry weight and total dry weight of *Helianthus maximiliani* increased at a NO_3–N concentration of 20 mg/L at both temperatures (Fig. 6, A and B).

Physiological response to water temperature and nitrogen concentration

Water temperature and N concentration significantly affected SPAD and CCI of *Asclepias speciosa*. High water temperature and higher NO_3–N concentrations (20 and 30 mg/L) had the highest SPAD and CCI. All combinations of N treatment and lower water temperature had the lowest SPAD and CCI (Table 3). However, there was effect of temperature and nitrogen concentration on the greenness index (G) and total chlorophyll (CPHLT). In contrast to *Asclepias speciosa*, water temperature and NO_3–N concentration did not affect SPAD, CCI, CPHLT, or G in *Helianthus maximiliani* (data not shown).

Table 3
Soil Plant Analysis Development (SPAD) index and Chlorophyll Content Index (CCI) for *Asclepias speciosa* (showy milkweed) in response to different water temperatures and nitrogen concentrations. Mean separations were carried out using the Tukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

Treatments	SPAD	CCI
75°F + 10 mg/L	17.0 ± 0.6^{b}	5.2 ± 0.1^{b}
75°F + 20 mg/L	17.0 ± 0.7^{b}	6.1 ± 0.1^{b}
75°F + 30 mg/L	17.7 ± 0.2^{b}	5.6 ± 0.1^{b}
85°F + 10 mg/L	16.8 ± 0.2^{b}	5.1 ± 0.1^{b}
85°F + 20 mg/L	21.7 ± 3.5^{ab}	8.6 ± 0.1^{ab}
85°F + 30 mg/L	30.9 ± 4.3^{a}	15.6 ± 0.1^{a}
Significance test (*p*-value)		
Temp	0.006^{***}	0.015^{**}
Nitrogen	0.014^{**}	0.037^{*}
Temp×Nitrogen	0.029^{*}	0.040^{*}

NS, *, **, *** denotes significant at p < 0.05, 0.01, or 0.001, respectively.

Tissue nitrate nitrogen concentration and total nitrogen uptake in response to temperature and nitrogen concentration

Increasing water temperature from 75 to 85 F decreased plant tissue NO_3–N content in both species at 10 mg/L N for *Helianthus maximiliani* and at 10 and 20 mg/L N for *Asclepias speciosa.* However, at lower N concentrations tissue NO_3–N concentration was similar for both species (Figs. 7 and 8).

An interactive effect of water temperature and N concentration was observed in both species. Tissue NO_3–N content of *Asclepias speciosa* was highest at the low water temperature and all levels of N concentration and was similar to 85 F and 30 mg/L N concentration. However, decreasing N concentration below 30 mg/L at 85 F decreased tissue N concentration (Fig. 7). Similarly, in *Helianthus maximiliani*, all interactions of water temperature and N concentration, except for 85 F and 10 mg/L N, resulted in higher tissue NO_3–N contents (Fig. 8).

Average tissue NO_3–N accumulation by *Asclepias speciosa* increased when nitrogen concentration was increased from 10 to 20 mg/L or 30 mg/L and at 85° F water temperature. However, for plants grown at 75° F, increasing NO_3–N concentration did not increase NO_3–N accumulation in plant tissue (Fig. 9).

For *Helianthus maximiliani*, the N concentration of 20 mg/L resulted in the highest NO_3–N accumulation in plant tissue. The interaction of water temperature and N concentration did not affect NO_3–N accumulation in plant tissue.

Plant water consumption

Increasing water temperature increased water consumption (water loss in tank) by both species (Fig. 12, A and B). *Asclepias speciosa* plants receiving 20 mg/L N concentration consumed more water at the high temperature compared to 10 and 20 mg/L N concentrations. At the lower water temperature, however, the response was reversed and plants receiving 20 mg/L N concentration consumed the least amount of water. *Helianthus maximiliani* plants grown under the lower water temperature had comparable water consumption across all N concentrations, but plants grown under the high water temperature had higher water consumption with a maximum of 53.7 liter observed at 20 mg/L N.

Nitrogen loss from tanks

Tissue NO_3–N concentration of *Asclepias speciosa* was highest at 75° F and 10 mg/L N and was similar to 85° F and 30 mg/L N concentration. However, decreasing N concentration below 30 mg/L at 85° F decreased tissue NO_3–N concentration (Table 4). Total NO_3–N accumulation was dependent on tissue dry weight and NO_3–N concentration was highest at treatments of 85° F and 30 and 20 mg/L N and 75° F, 30 and 10 mg/L.

For *Helianthus maximiliani*, tissue NO_3–N concentrations were similar for all experimental treatments except for the 85° F and 10 mg/L N concentration treatment and NO_3–N accumulation was the same across all treatments and N concentrations (Table 5).

Table 4
Tissue nitrate-nitrogen concentration and total nitrate-nitrogen accumulation of *Asclepias speciosa* (showy milkweed) in response to different water temperatures and nitrogen concentrations. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

Temp. (°F)	Nitrate Nitrogen concentration (mg/L)	Total amount of water applied (L)	Total Nitrate -Nitrogen applied (mg)	Nitrate-Nitrogen in tissue (mg/kg of dry weight)	Total nitrate nitrogen accumulation (mg)
75	10	43.48	434.8	1550 ± 280.29^{a}	5.43 ± 0.94^{ab}
75	20	37.08	741.6	1400 ± 70.59^{ab}	4.20 ± 1.27^{b}
75	30	51.1	1533	1033.33 ± 54.6^{ab}	7.08 ± 0.61^{ab}
85	10	48.45	484.5	416.67 ± 16.33^{c}	3.50 ± 0.46^{b}
85	20	53.6	1072	933.33 ± 41.31^{bc}	9.31 ± 1.03^{a}
85	30	50.7	1521	1260 ± 153.35^{ab}	8.68 ± 1.95^{a}

Table 5
Tissue nitrate-nitrogen concentration and total nitrate-nitrogen accumulation of *Helianthus maximiliani* (maximilian sunflower) in response to different water temperatures and nitrogen concentrations. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

Temp. (°F)	Nitrate-Nitrogen concentration (mg/L)	Total amount of water applied (L)	Total Nitrate-Nitrogen applied (mg)	Nitrate-Nitrogen in tissue (mg/kg of dry weight)	Total nitrate nitrogen accumulation (mg)
75	10	41.05	410.5	8800 ± 602.2^{a}	19.15 ± 5.1^{a}
75	20	41.75	835	9050 ± 585.2 ^{a}	38.43 ± 13.81^{a}
75	30	39.39	1181.7	8400 ± 535.5 ^{a}	19.91 ± 6.15^{a}
85	10	44.92	449.2	4766.67 ± 198.7^{b}	11.60 ± 3.36^{a}
85	20	47.62	952.4	8600 ± 515.9 ^{a}	37.76 ± 9.76 ^{a}
85	30	40.45	1213.5	7333.3 ± 416.2 ^{a}	11.25 ± 3.49 ^{a}

Discussion

Morphological response to nitrogen concentration and water temperature

Plant growth and photosynthesis are most sensitive to temperature changes. Exposure of plant roots to temperature above 95°F could damage the photosynthetic process by destroying components of PSII and PSI (Yamori et al. 2022). This would consequently decrease the concentration of dissolved oxygen in the nutrient solution, thereby affecting plant growth (Suzuki et al. 2008). The increased root respiration at high temperatures would lead to a decrease in leaf photosynthetic rate, further impacting plant growth (Klock et al. 1997). The allocation of nutrients between shoot and root systems can be influenced by temperature changes, impacting nutrient acquisition and carbon fixation in plants. In our study, increasing water temperature from 75° to 85°F increased growth for both taxa. Similar to our study, optimum temperature between 77 to 86°F significantly increased root branching increasing higher uptake of nutrients promoting plant growth in three subtropical plant species *Corchorus capsularis*, *Mimosa sepiaria*, and *Ormosia glaberrima* (Luo et al. 2020).

Increasing water or root zone temperatures can increase enzymatic activity in plants, facilitating nutrient absorption and utilization and increasing plant growth (Levine et al. 2023). The *Lactuca sativa* (lettuce) plant grown in 86°F root temperature showed maximum plant growth, higher CO_2 assimilation rate, stomatal conductance, and electron transport rate (ETR) compared to grown in 77°F (Yamori et al. 2022). Increasing N concentration can also increase growth by promoting cell expansion, facilitating chlorophyll synthesis and increasing energy transfer during photosynthesis (Zarrinmehr et al. 2020).

Similar to our study, a study of *Caltha palustris* by Lindborg et al. (2021) found increases in plant height, leaf area, and numbers of leaves and roots when the root zone temperature was increased even by 1°F. However, for *Sphagnum* sp. increases in temperature did not affect growth (Zarrinmehr et al. 2020). Therefore, the response of root temperature to plant growth is species-dependent (Charles & Dukes 2009). Similar to *Helianthus maximiliani* in our study, increasing water temperature from 60° to 75°F for three wetland plants (*Potamogeton lucens*, *Vallisneria spiralis*, and *Elodea nuttallii*) increased shoot and root biomass and growth rate (Szabó et al. 2020; Zhang et al. 2019) and increasing air temperature from 75° to 86°F in *Asclepias syriaca* increased plant growth by 32%. The increase in temperature increased the photosynthesis rate, metabolic rate, and plant hormone concentrations such as auxin and cytokinin, leading to higher shoot growth (Couture et al. 2015). The optimal temperature range for photosynthesis in submerged wetlands plants is between 77° and 89°F (Barko et al. 1982; Pedersen et al. 2013). Therefore, at 75° F, growth is reduced as compared to 85°F as seen in our study. In our study, a water temperature of 85°F improved growth for both *Helianthus maximiliani* and *Asclepias speciosa*. For *Asclepias speciosa*, though, the same effect was achieved by increasing N concentration to 30 mg/L.

Increasing N concentration to 30 mg/L at the lower water temperature also increased stem growth. For *Asclepias*, increasing N concentration to 30 mg/L would compensate for the reduction in temperature. However, at 10 and 20 mg/L, the N uptake would decrease due to the lower temperature. In contrast, for *Helianthus*, nitrogen concentration had no effect on the growth and its tissue N concentration and accumulation were higher compared to *Asclepias*. This suggests that *Helianthus* are better at N uptake capabilities compared to *Asclepias.*

Physiological response to temperature and nitrogen concentration

Increasing water temperature and N concentration did not increase SPAD index, CCI, G, or total chlorophyll content in *Helianthus maximiliani* which is consistent with Zhang et al.'s (2008) study of wheat cultivar 'Yumai 34' and Luo et al.'s (2023) study of tomato (*Solanum lycopersicum*). This is because the tissue NO_3–N concentration in this study was similar for all experimental treatments, except 85° F and 10 mg/L N, and N is responsible for chlorophyll formation and SPAD index.

For *Asclepias speciosa*, all treatments had similar SPAD indices and CCI, except for the 85° F and 20 and 30 mg/L N concentrations (Table 3) which had highest SPAD and CCI. Increased temperature and increased N concentration led *Asclepias* to take up more nitrogen as evident in our N accumulation graph. The increase in tissue N content increases chlorophyll levels in leaves, explaining the increase in SPAD and CCI value at higher temperature and higher N concentration. Additionally, this observation suggests that *Asclepias* are less efficient in N uptake compared to *Helianthus*. Increase in tissue NO_3–N concentration may increase metabolic activity and the uptake of water and nutrients at higher water temperatures and N concentrations (Levine et al. 2023; Luo et al. 2020) and may also increase leaf greenness and chlorophyll content.

Generally, N level strongly correlates with chlorophyll content and decreasing the amount of N applied reduces photosynthetic efficiency and chlorophyll content (Bassi et al. 2018; Prsa et al. 2007; Wu et al. 2019). For example, a study by Szabó et al. (2020) of two submerged plants (*Elodea canadensis* and *Elodea nuttallii*) showed increased chlorophyll concentrations with increased N concentrations.

Biomass accumulation in response to nitrogen concentration and temperature

At harvest, *Asclepias speciosa* had accumulated higher total biomass compared to *Helianthus maximiliani* as indicated by total fresh (data not shown) and dry weights (Tables <link rid="tb3">3</link>–4 and 3–5). Nitrogen concentration and water temperature both effected root and total dry mass of *Asclepias speciosa*, however, only water temperature increased shoot dry weight and there was no effect of N concentration. Increasing water temperature can increase metabolic activity in plants, leading to higher water demand and growth (Zarrinmehr et al. 2020) which we observed in root, shoot, and total dry weights. Kong et al. (2023) observed a similar response by two leafy greens (*Lactuca sativa* 'Cegolaine' lettuce and *Brassica rapa* 'Petite star' pak choy) where increasing water temperature from 70 to 86 F increased shoot fresh and dry weights by 30% and 53% in *Lactuca sativa* and *Brassica rapa* and by 22% in Pak choy. In addition, an increase in plant growing temperature increases leaf expansion, improving photosynthesis and resulting in higher biomass production (Kong et al. 2023). Studies by Lin et al. (2010) and Baldwin et al. (2014) reported increases in the biomass of wetland plants such as *Lythrum salicaria* and *Bidens* sp. as water temperature was increased. In this study, increasing N concentration to 30 mg/L even at the lower water temperature increased root mass as there was more nitrogen to absorb.

For *Helianthus maximiliani*, water temperature and N concentration did not affect shoot dry weight, but root and total dry weight was higher at the 20 mg/L N concentration. This may be because the optimum N concentration was 20 mg/L N and above or below that level there would be deficiency or toxicity. We did not, however, observe any visual signs of N deficiency or toxicity in this study. *Helianthus maximiliani* grows more slowly than *Asclepias speciosa*, which explains why any potential differences may not have been observed yet due to the short growing time. Also, water temperature requirements were optimal for *Helianthus* in our study; hence, there was no effect of temperature.

Tissue nitrate nitrogen and total plant uptake in response to temperature and nitrogen concentration

For *Asclepias*, tissue NO_3–N concentration was highest at lower temperature and at 30 mg/L under higher temperatures, showing a contrast in accumulation patterns. When compared with growth data, it becomes evident that at higher temperature plant biomass increased, resulting in dilution of N concentration but at lower temperature the mass was lower, leading to an increase in tissue N concentration. Our finding is similar to findings reported by Zheng et al. (2016), where NO_3^- removal efficiency increased with increasing temperature in polluted stream and a study by Alexandre et al. (2020) of *Zostera marina* (seagrass), where increasing water temperature from 60□ to 77□ F resulted in 50% higher NO_3^- uptake by plants. A study by Kaldy (2014) also found higher NO_3^- uptake with increasing water temperature. As plant biomass increases, the available N is distributed across a larger mass of plants. The nutrients become more dispersed, reducing their concentration in units of biomass. Kong et al.'s (2023) study of lettuce and Pak choy found decreased shoot NO_3^- contents as water temperature increased. In this study, higher water temperature led to faster and higher growth rates, resulting in lower tissue NO_3- content because of increased biomass, similar to the studies of Reich & Oleksyn (2004) and Zhang et al. (2019).

The total tissue NO_3–N concentration of *Helianthus maximiliani* did not increase with increasing water temperature or N concentration in this study except for 85° F and 10 mg/L N. This might be because *Helianthus* are more efficient at N uptake at wide range of nutrient and temperature conditions. However, total NO_3–N accumulation was highest at the 20 mg/L N concentration (independent of temperature) which is because the growth and the dry weight was highest at the same N concentration and higher the biomass higher is the N accumulation. Also, the optimum growing temperature for *Helianthus maximiliani* is between 72° and 86° F and the temperatures we provided were optimum thus we did not see any effect of water temperature. However, N concentration did affect NO_3–N accumulation and the optimum N concentration for *Helianthus maximiliani* may be 20 mg/L.

For both species in this study, NO_3–N accumulation did not exactly follow a similar pattern to tissue NO_3–N concentration, as it was influenced by tissue weight. Therefore, even though two plants might have similar NO_3–N tissue concentrations, the total accumulation would be higher in a plant with higher biomass. When assessing N removal, it is crucial to consider total accumulation compared to tissue concentrations.

Correlation analysis

There was a positive correlation between total NO_3–N accumulation and plant dry weight for both *Asclepias speciosa* and *Helianthus maximiliani* in this study. This indicates a higher increase in dry weight will increase NO_3–N accumulation in both species and vice versa. Nitrogen is a primary plant nutrient that is required for photosynthesis and production of carbohydrates and increasing N applications can increase plant dry weight. In general, *Helianthus maximiliani* had higher NO_3–N accumulation at the same N concentration as *Asclepias speciosa*. Although dry biomass of *Helianthus maximiliani* was lower than *Asclepias speciosa*, the uptake of NO_3–N was very high in *Helianthus maximiliani* as compared to *Asclepias speciosa*. Therefore, even with less biomass than *Asclepias speciosa, Helianthus maximiliani* can take up and accumulate higher quantities of NO_3–N. Usually, increased biomass results in high demand for nutrients (Reay et al. 1999; Wang et al. 2019). However, at lower dry weight *Helianthus maximiliani* had high NO_3–N accumulation compared to *Asclepias speciosa*, indicating greater efficiency in NO_3- uptake. This might be important for NO_3–N removal from poor quality water as species with lower growth rates may be more efficient at NO_3–N uptake compared to species with higher growth rates.

Conclusion

Both plants in this study grew well in the FTW system and took up a significant amount of NO_3–N, indicating that they may be candidates for NO_3- removal as FTW. Increasing water temperature increased growth for both species and increased dry weight for *Asclepias speciosa*. However, for *Helianthus maximiliani*, increasing water temperature had a lesser effect on total dry weight. Therefore, increasing water temperature may increase plant growth, but its effect is species-specific. Neither water temperature nor N concentration affected CCI and G in this study. The accumulation of NO_3–N was higher in *Helianthus maximiliani* at 20 mg/L N and either water temperature. However, in *Asclepias speciosa*, the accumulation of NO_3–N was higher at the higher water temperature of 85° F and 20 mg/L N. Therefore, N accumulation may be either dependent on water temperature or N concentration depending on species and higher N concentration does not always lead to higher NO_3–N accumulation in plants. However, for a particular species, as plant growth increases, total NO_3–N accumulation also increases. This does not mean, however, that larger plants always accumulate more NO_3–N as uptake efficiency differs among species. The results of this study indicate that *Helianthus maximiliani* and

Asclepias speciosa may both be used for the absorption of NO_3–N in FTW, although *Helianthus maximiliani* accumulated more NO_3–N than A*sclepias speciosa.*

Declarations

Ethical Approval

Not applicable in our research

Consent to Participate

Not applicable in our research

Consent to Publish

Not applicable in our research

Funding

This project was supported by Utah Agricultural Experiment Station (UAES) fund project number UTA01631 and partly by Western SARE grant WPDP22-014 awarded to Dr. Shital Poudyal.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization by Saroj Burlakoti, Kelly Kopp, Lance Stott and Shital Poudyal; Methodology by Saroj Burlakoti; software and data analysis Saroj Burlakoti and Shital Poudyal; validation by Kelly Kopp, Lance Stott and Shital Poudyal; writing—original draft preparation by Saroj Burlakoti; writing—review and editing by Kelly Kopp, Lance Stott and Shital Poudyal; supervision by Kelly Kopp, Lance Stott and Shital Poudyal; funding acquisition by Shital Poudyal

Acknowledgments

We thank Paul Harris, Anju Chaudhary and Jenny Koller for assisting with the experimental setup, plant harvesting, and data collection.

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Figures

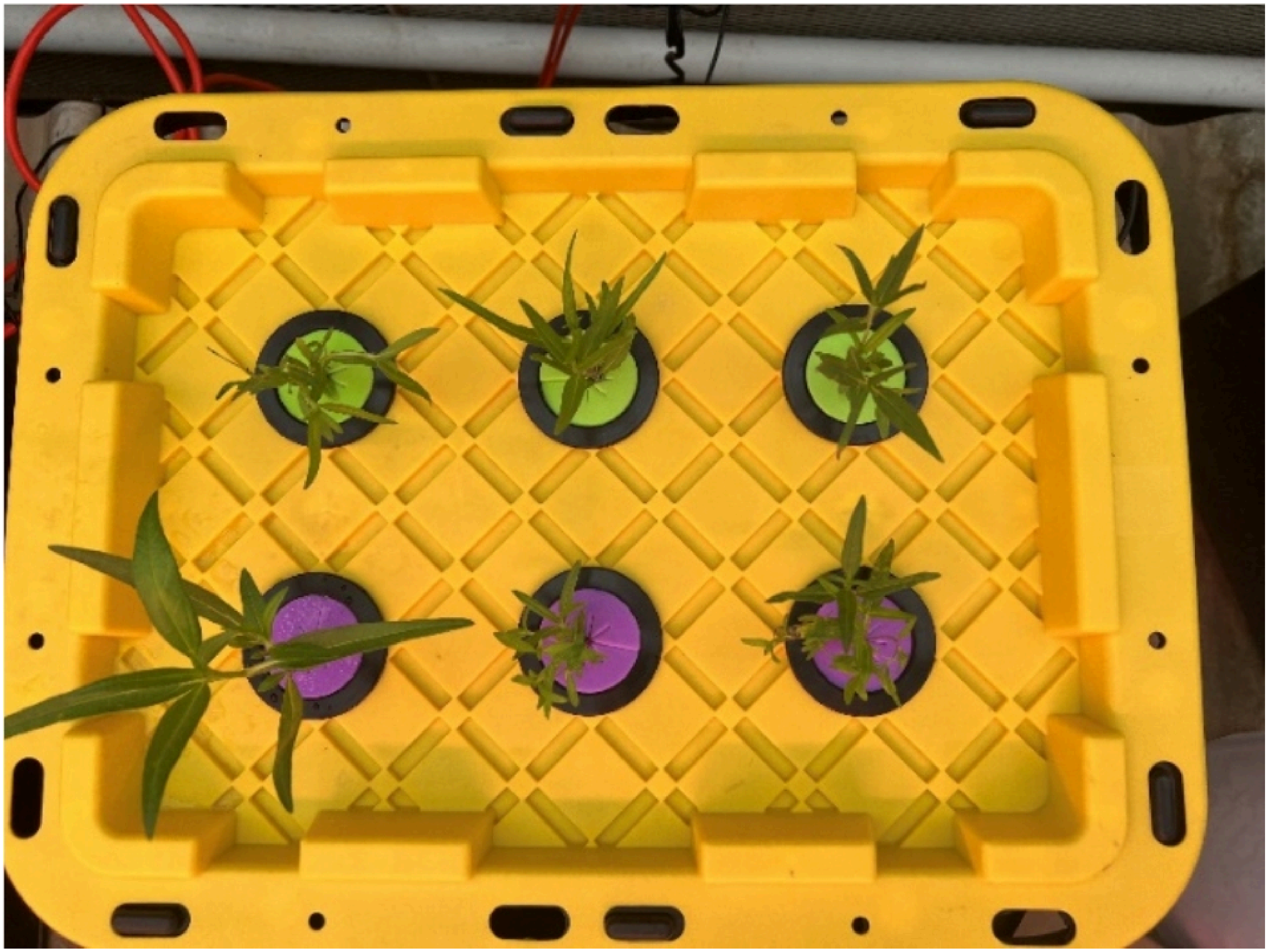

Figure 1

Experimental setup of plants in a single tank (left) and overall complete experimental arrangement (right)

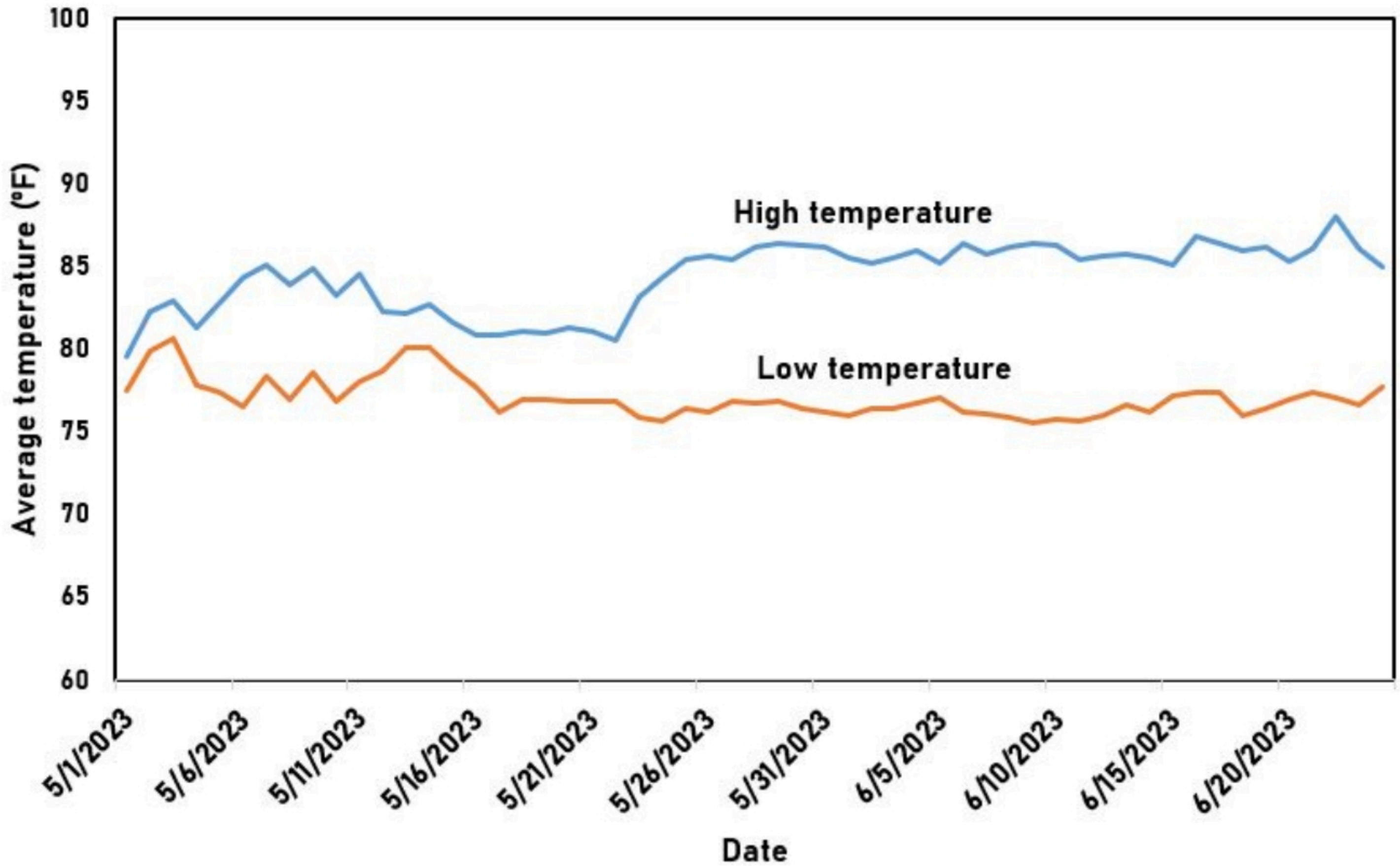

Figure 2

Temperature recorded by temperature data logger during the course of the experiment.

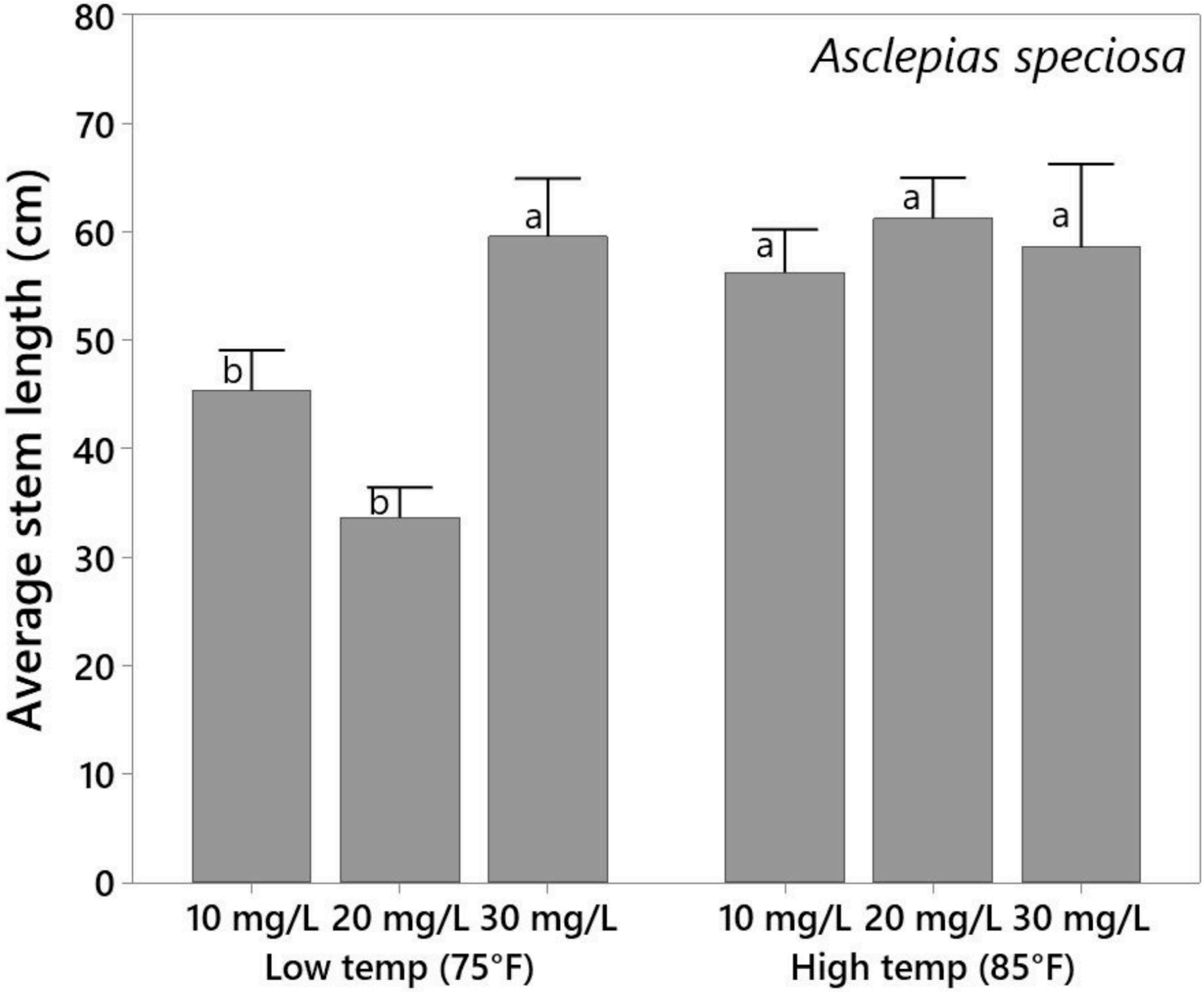

Figure 3

Average stem length (cm) for *Asclepias speciosa* (showy milkweed) in response to different temperature and nitrogen concentrations. Standard errors of the means are denoted by vertical 'T' lines. Mean separations for each species were carried out using the Tukey post-hoc test ($P < 0.05$).

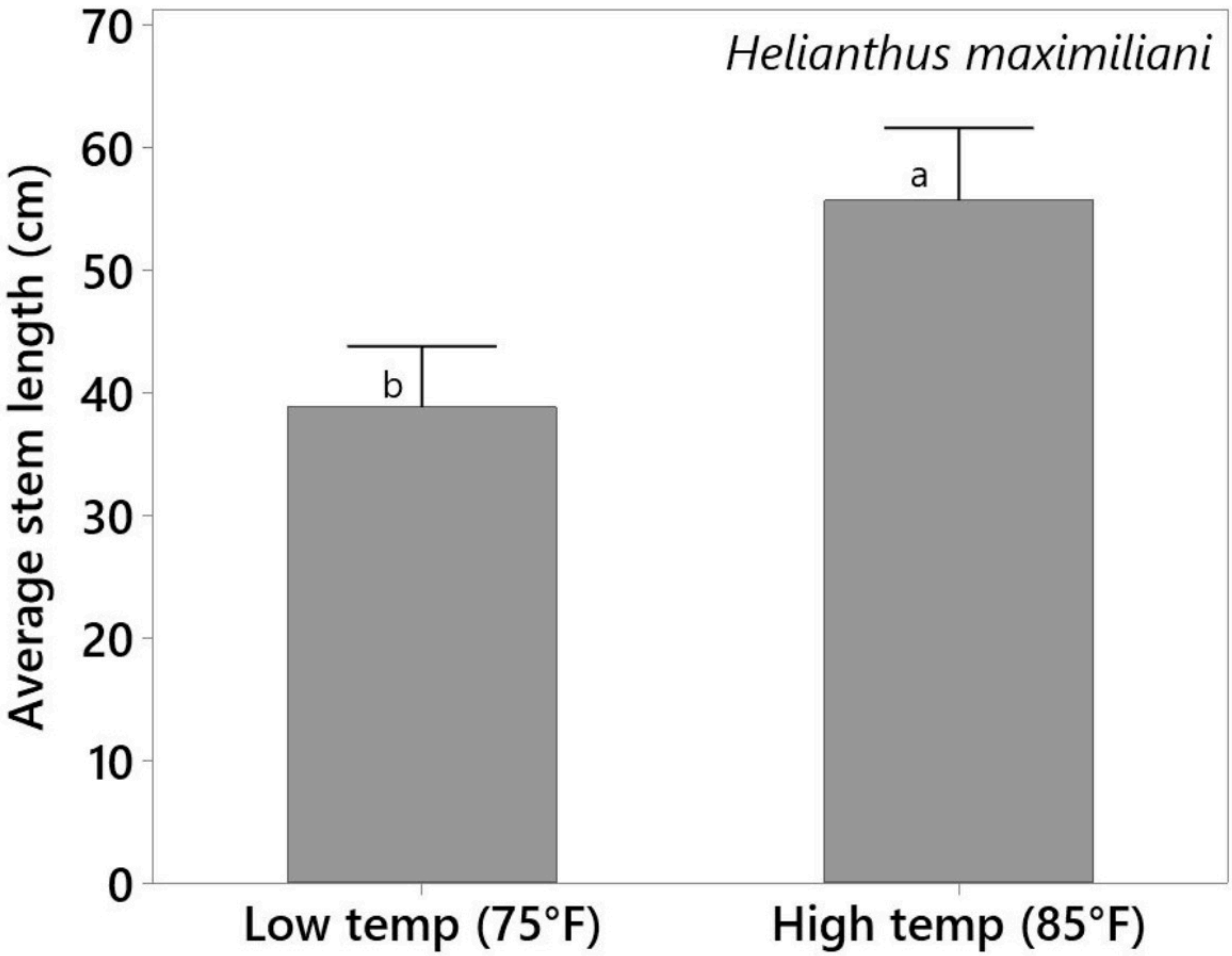

Figure 4

Average stem length (cm) for *Helianthus maximiliani* (maximilian sunflower) in response to water temperature. Standard errors of the means are denoted by vertical 'T' lines. Mean separations for each species were carried out using Tukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

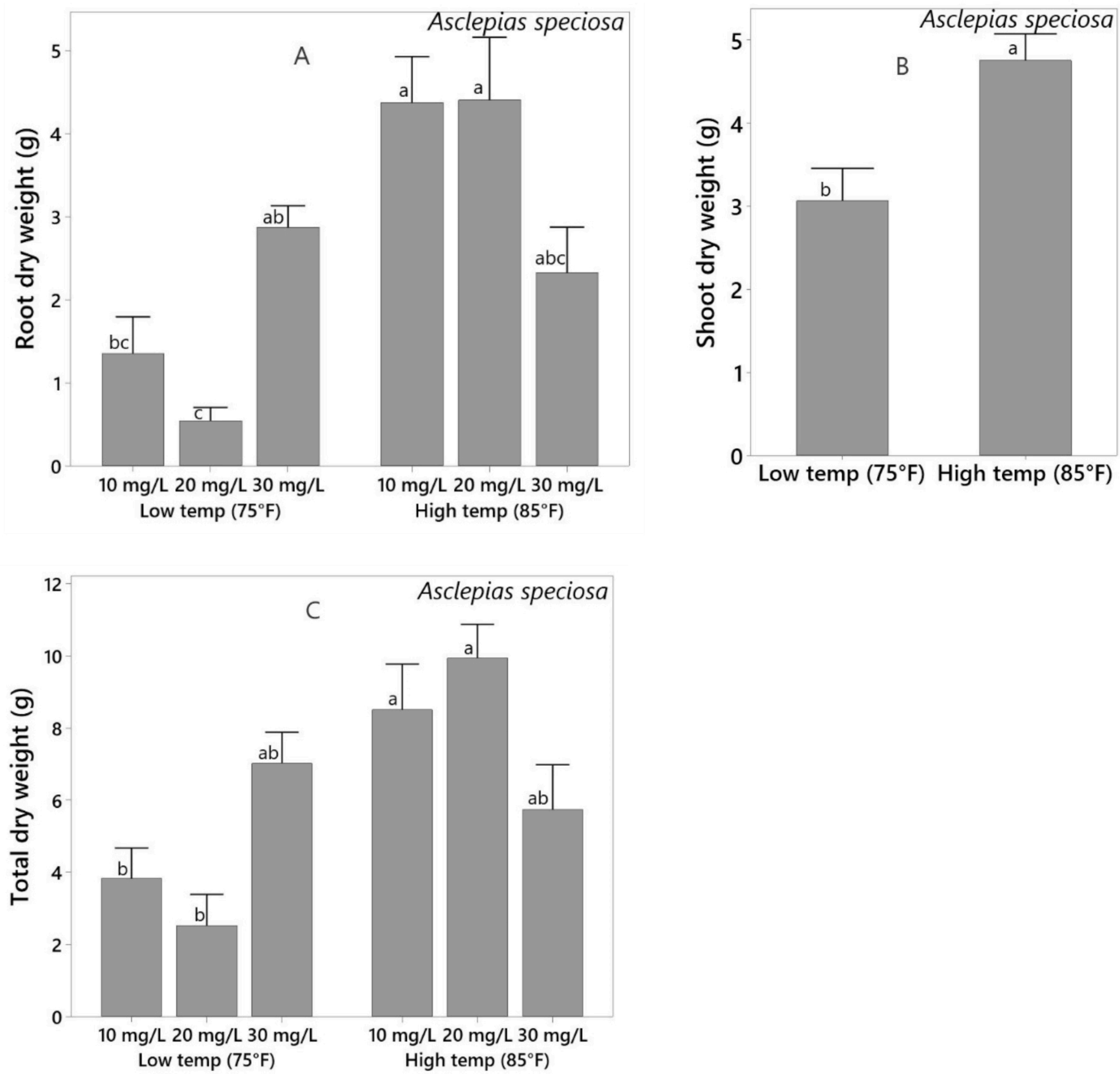

Figure 5

Dry weight (shoot, root and total) for *Asclepias speciosa* (showy milkweed) in response to different water temperatures, nitrogen concentrations, and their interactions. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

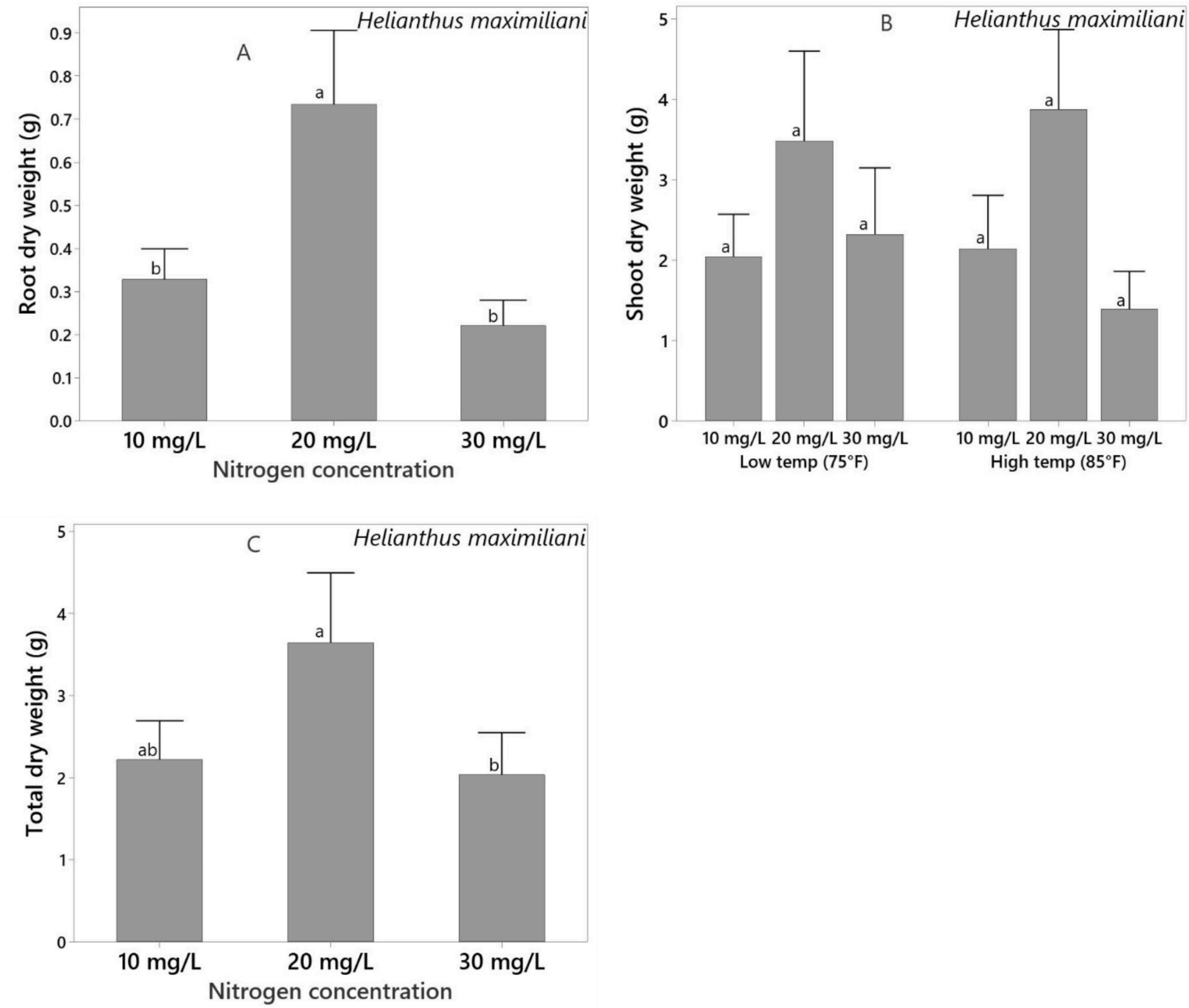

Figure 6

Shoot, root, and total dry weights of *Helianthus maximiliani* (maximiliani sunflower) in response to different water temperatures and nitrogen concentrations. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

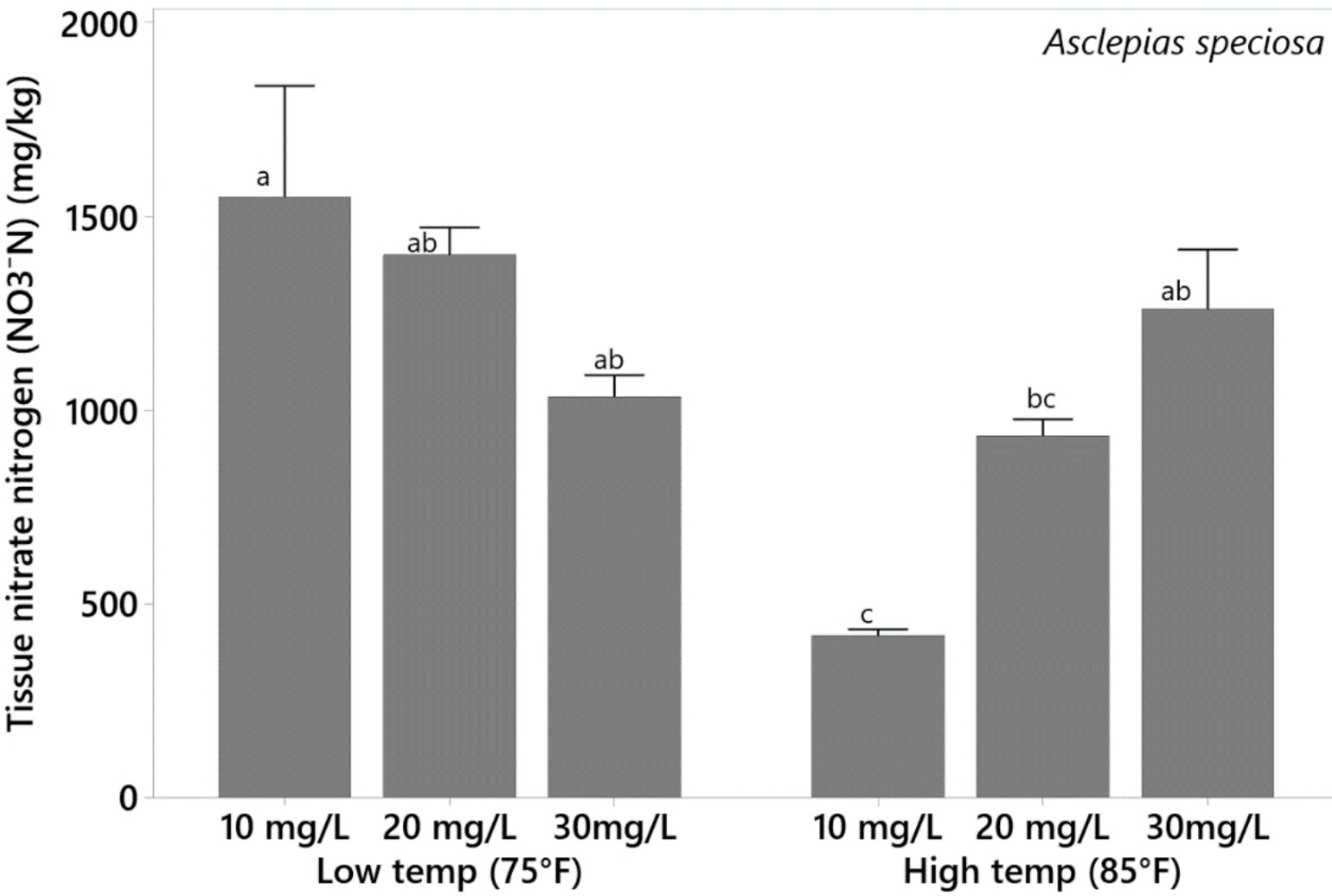

Figure 7

Tissue nitrate nitrogen content (mg/kg of dry weight) for *Asclepias speciosa*(showy milkweed) in response to different water temperature and nitrogen concentrations. Standard errors of the means are denoted by vertical 'T' lines. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

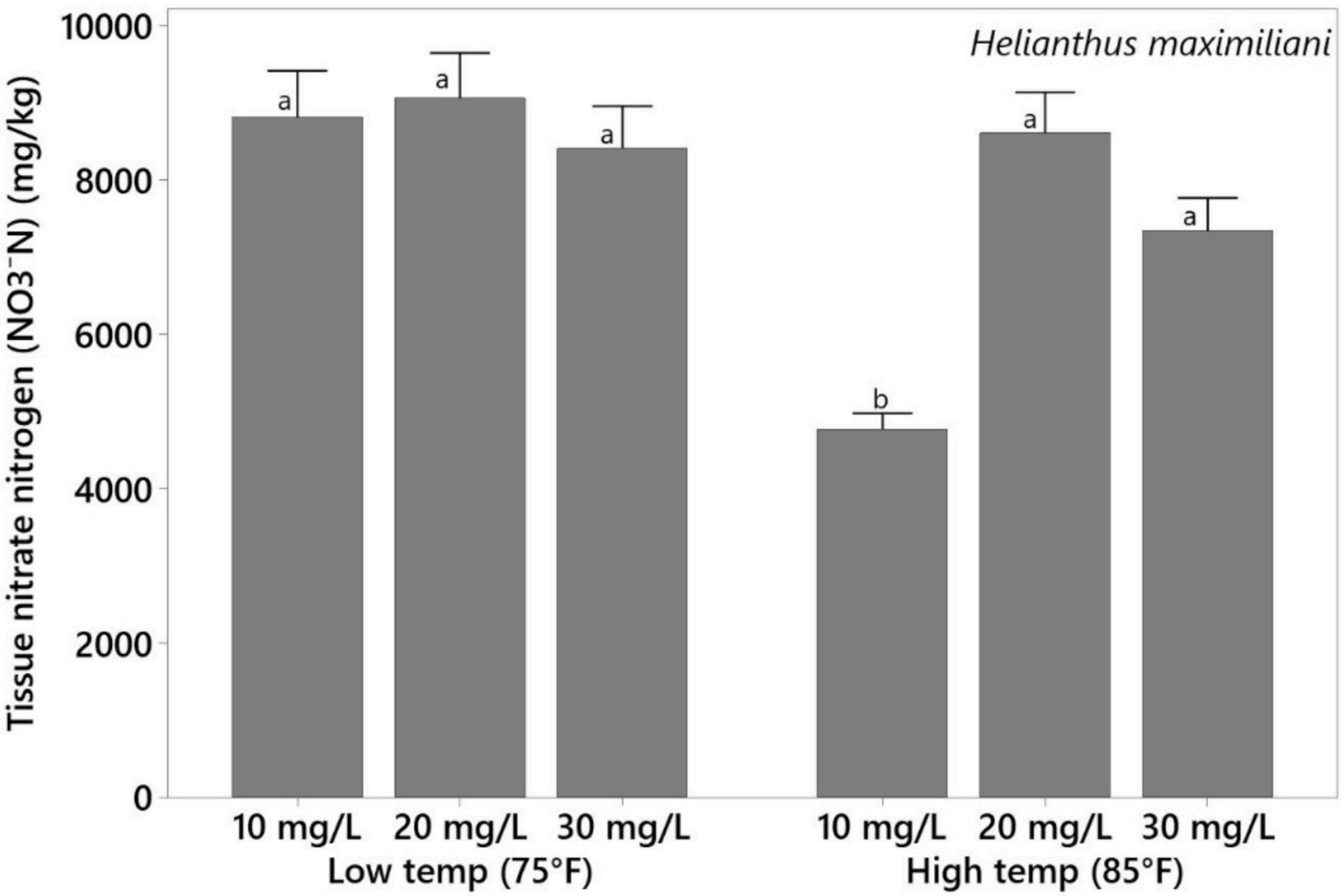

Figure 8

Tissue nitrate nitrogen content (mg/kg) of *Helianthus maximiliani* (maximilian sunflower) in response to the interaction of different water temperatures and nitrogen concentrations. Standard errors of the means are denoted by vertical 'T' lines. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

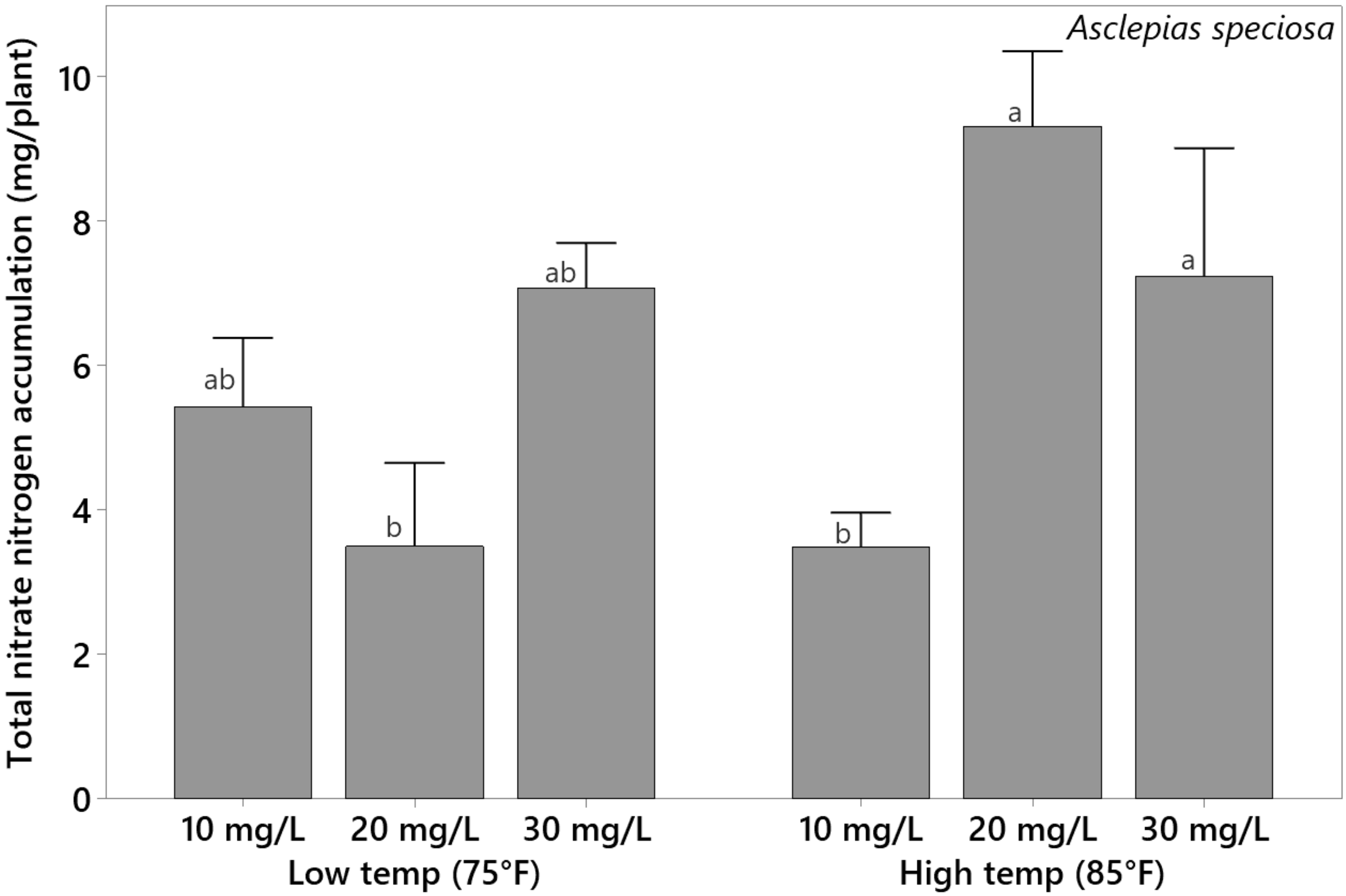

Figure 9

Total nitrate nitrogen accumulation (mg) for Asclepias speciosa (showy milkweed) in response to different water temperatures and nitrogen concentrations. Standard errors of the means are denoted by vertical 'T' lines. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

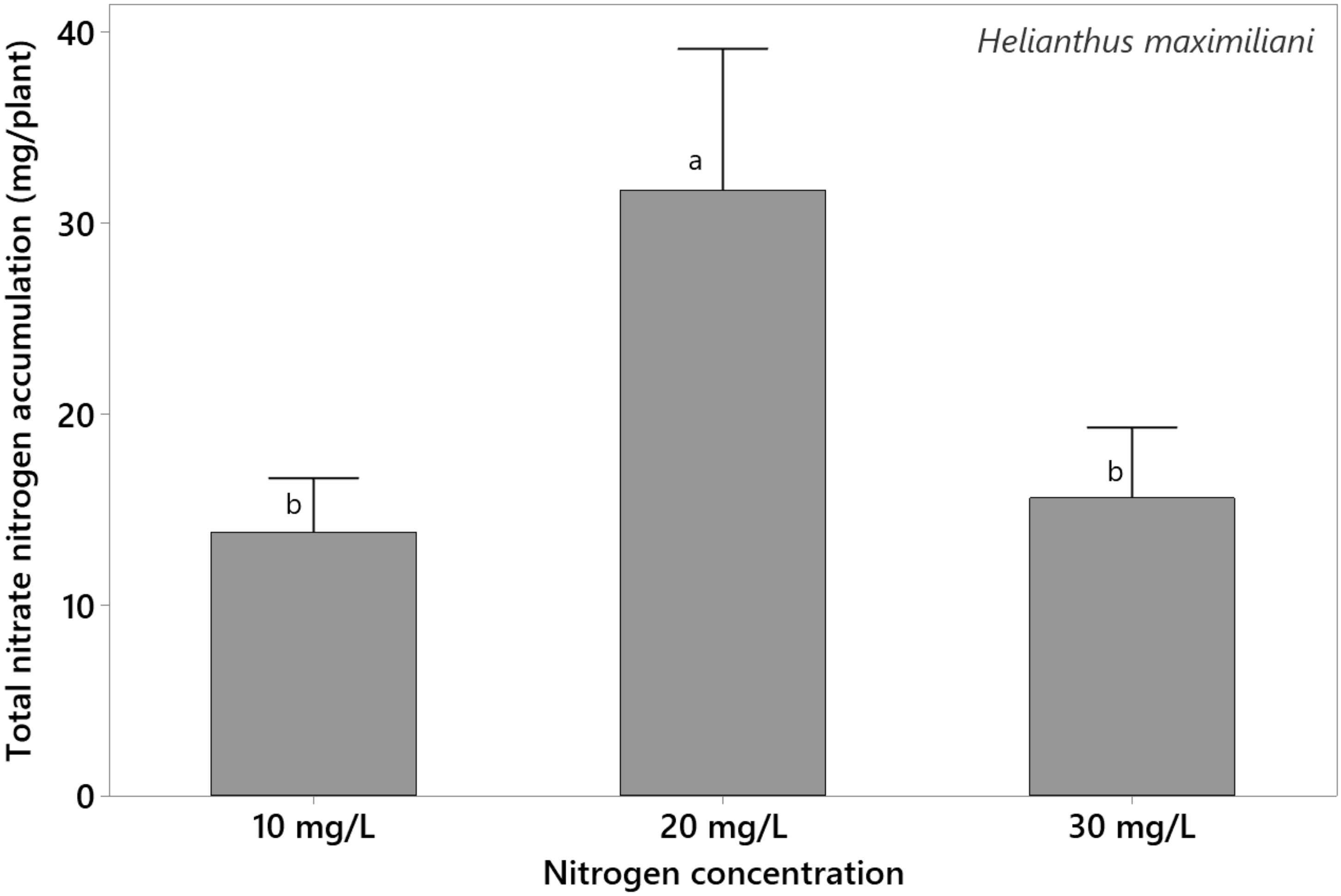

Figure 10

Total nitrate nitrogen accumulation (mg) of *Helianthus maximiliani* (maximilian sunflower) in response to different nitrogen concentrations. Standard errors of the means are denoted by vertical 'T' lines. Mean separations were carried out using theTukey post-hoc test ($p < 0.05$). Means with the same letters are not significantly different from one another ($p < 0.05$)

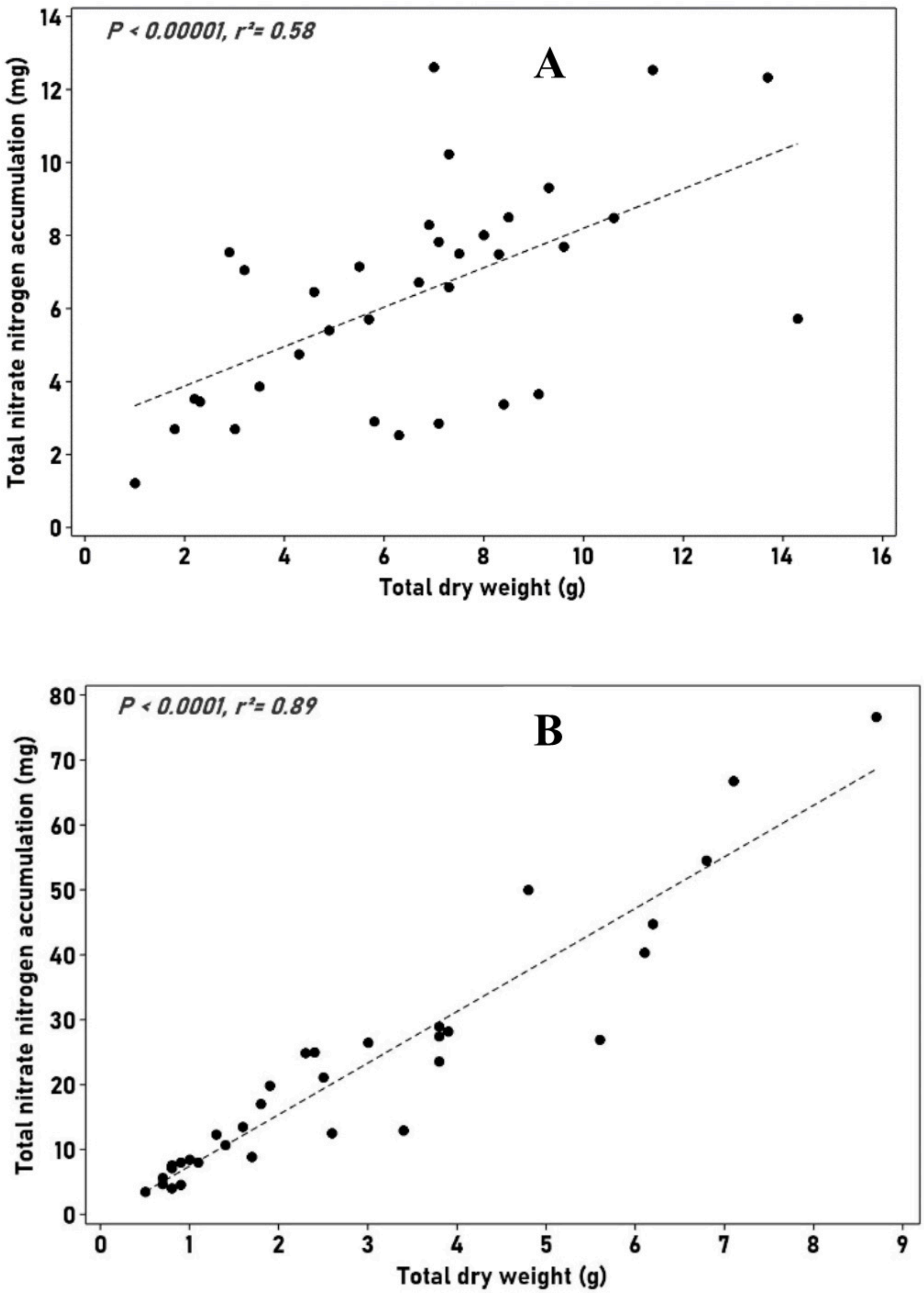

Figure 11

Correlations between total plant dry weight (g) and total nitrate nitrogen accumulation (mg) in *Asclepias speciosa* (showy milkweed) (A) and *Helianthus maximiliani* (maximilian sunflower) (B)

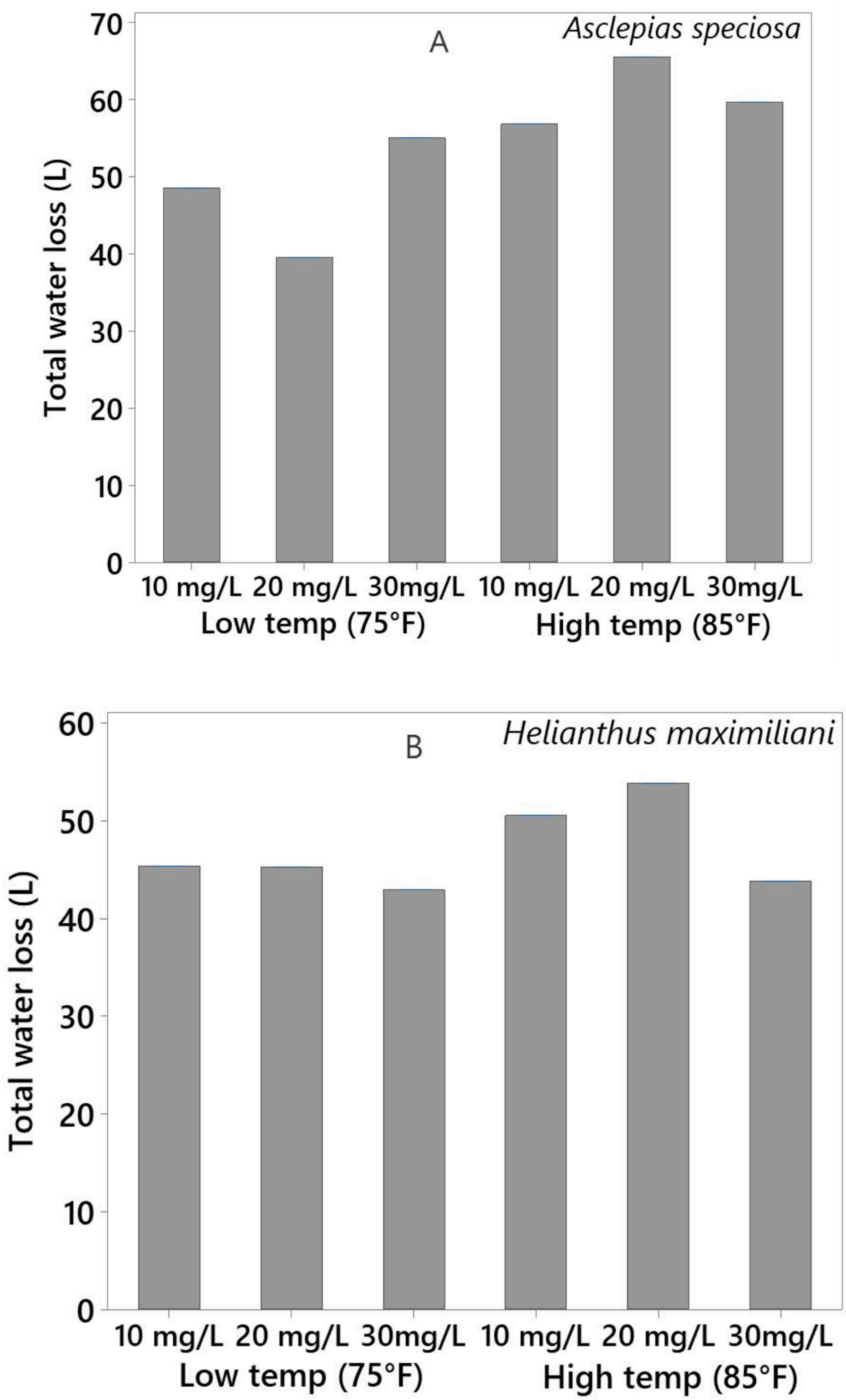

Figure 12

Total water loss of *Asclepias speciosa* (showy milkweed) (A) and *Helianthus maximiliani*(maximiliani sunflower) (B) in response to different water temperatures and N concentrations during treatment period.