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# Hydraulic Redistribution in Slender Wheatgrass (*Elymus trachycaulus* Link Malte) and Yellow Sweet Clover (*Melilotus officinalis* L.): Potential Benefits for Land Reclamation

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**Abstract:** Hydraulic redistribution (HR) by plant roots can increase moisture content in the dry, mostly upper, parts of the soil. HR helps maintain the viability of fine roots, root hydraulic conductivity, microbial activity and facilitate nutrient uptake. Plants can supply water to other surrounding plants by HR under drought conditions. In oil sands reclamation areas in Northeastern Alberta, Canada, reconstructed soils commonly suffer from the problems of drought, high pH, salinity, and compaction, which often impact revegetation success. In this study, we investigated the HR potential of two herbaceous plants that are frequently present in oil sands reclamation sites: slender wheatgrass (*Elymus trachycaulus* Link Malte) and yellow sweet clover (*Melilotus officinalis* L.), using a vertically split-root growth setup and treatments with deuterium-enriched water. Our objective was to test the potential benefits of HR on drought responses of seedlings of the commonly used plant species for oil sand reclamation, balsam poplar (*Populus balsamifera* L.), when these plants were grown together under controlled environment conditions. We found that both wheatgrass and yellow sweet clover could redistribute water in the upward and downward directions. However, the amount of water released by the roots was not sufficient to alleviate the effects of drought stress on the associated balsam poplar seedlings. Longer-term field studies should be carried out in order to examine, under different environmental conditions, the potential benefits of HR in these herbaceous plants to the establishment and growth of other plant species that are used for land reclamation.

**Keywords:** hydraulic lift; drought; plant water relations; land reclamation

## 1. Introduction

Hydraulic redistribution (HR) is the water movement driven by water potential gradients from wet soils to dry soils via plant roots [1]. HR mostly occurs at night when stomatal conductance and transpiration are low, thus the root xylem water potential increases and the roots may release water to the soil [1,2]. HR includes upward [2], downward [3], and lateral [4] fluxes of water along the water potential gradients in soil profiles. Increased moisture content in drier soils by HR is supposed to help maintain the viability of fine roots [5], root hydraulic conductivity [6], microbial activity [7], and facilitate in nutrient uptake [8].

Several studies have reported that woody plants can supply hydraulically redistributed water to surrounding grasses [9–11] and forage plants can supply water to neighboring crops and function as biological irrigation systems [12,13]. In natural ecosystems, deep-rooted trees and shrubs often lift

water from deep moist soils to shallow rooted grasses [2]. Since planted tree seedlings have shallow root systems, they could also potentially benefit from HR of the deeper-rooted herbaceous plants. However, HR by herbaceous plants has mainly been studied in agroecosystems [13–15].

Oil sands mining in Northeastern Alberta, Canada, disturbs vast areas of the boreal ecosystem, since it involves complete removal and reconstruction of landforms. The impacts of oil sands mining on the environment are mitigated through reclamation, reforestation and closure activities. Plants in the oil sands reclamation areas are commonly faced with high soil pH, soil compaction and drought conditions [16,17]. These soil factors commonly affect the establishment and growth of young seedlings following planting [18,19] and impact root colonization by mycorrhizal fungi, as well as water and mineral nutrient uptake by plants [20–22].

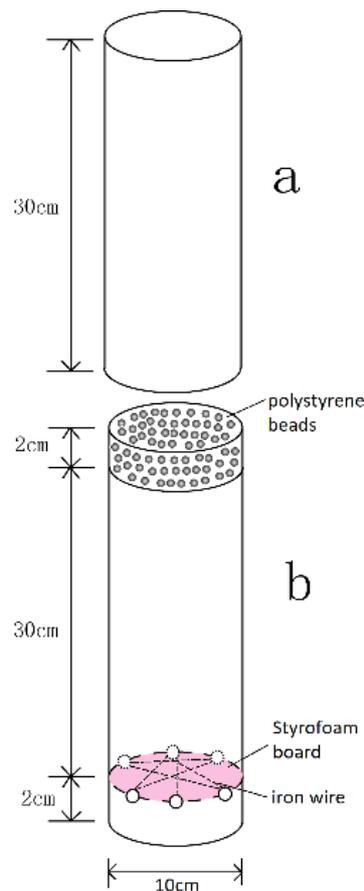
In the oil sands reclaimed sites, agronomic grasses and legumes are planted before trees to suppress soil erosion [19]. The potential of using HR of herbaceous plants to improve revegetation success in oil sands reclamation areas could be highly significant if there are HR plants that are suitable for the planting sites. In the present study, we used a vertically split-root pot culture to examine the HR capability of two deep-rooted herbaceous plants that are commonly used in reclamation practices: slender wheatgrass (*Elymus trachycaulus* Link Malte) and yellow sweet clover (*Melilotus officinalis* L.). Balsam poplar (*Populus balsamifera* L.) is among the dominant tree species in the boreal forest surrounding the oil sands mining areas and is commonly used for oil sands reclamation. The objective of this study was to determine whether the presence of wheatgrass and clover in the vicinity of balsam poplar seedlings could affect soil moisture content and potentially benefit the growth of balsam poplar seedlings through the HR processes.

## 2. Materials and Methods

### 2.1. Split-Root Setup and Plant Material

The split-root growth setup consisted of two polyvinyl chloride (PVC) tubes (10 cm in diameter); the upper one (part-a) was 30 cm long (volume 2.4 L), and the lower one (part-b) was 34 cm long (volume 2.7 L) (Figure 1). Holes were drilled at 2 cm from the end of the lower tube and iron wires were knitted in those holes to form a net supporting a 1 cm-thick Styrofoam board. The lower PVC tube was filled with 30 cm of commercial growing mix (sunshine professional growing mix 2.8 CU FT SS LA4, Sun Gro Horticulture, Seba Beach, AB, Canada), which consists of Canadian sphagnum peat moss, coarse perlite, dolomitic limestone, and long-lasting wetting agent. The top 2 cm of the lower tube was filled with polystyrene beads (3 mm in diameter). Then, the upper and lower tubes were sealed with adhesive tape and the upper tube was filled with the growing mix. The 2 cm layer of polystyrene beads prevented capillary water movement through the soil between the upper and lower PVC tubes.

Slender wheatgrass (*Elymus trachycaulus*) and yellow sweet clover (*Melilotus officinalis*) seeds were germinated and seedlings grown for two months in pots and then transplanted to the upper tube segment of the split-root growth setup. The plants were grown in a controlled environment growth room with 22/18 °C (day/night) temperature, 50 ± 10% relative humidity, and 16 h photoperiod (6:00 to 22:00) with 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) at the top of the seedlings, provided by the full spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). The plants were watered daily by adding water in the upper tube (part-a) until the water permeated through the lower tube (part-b), and were provided weekly with 2 g L<sup>-1</sup> 20-20-20 fertilizer (Agrium Advanced Technologies, Calgary, AB, Canada). The plants were grown for two months until the roots reached the bottom of the lower tube.



**Figure 1.** Schematic illustration of the vertical split-root growth system used in this study. Two 10 cm diameter polyvinyl chloride (PVC) tubes were stacked together and sealed with the tape. Holes were drilled 2 cm above the base of the lower tube and iron wires were knitted to form a net supporting a 1 cm-thick Styrofoam board with the soil on the top. The soil in the upper (a) and lower (b) tubes was separated by a 2 cm-thick layer of polystyrene beads to prevent upward water movement in the soil from the lower to the upper tube segment.

## 2.2. Experimental Design and Deuterium (D)-Enriched Water Treatments

Two independent experiments were carried out. In each of them, five plants per species were grown in the split-root setup for four months until the roots reached the growth mix in the lower tube segment. The soil volumetric water content was monitored with the time domain reflectometry (TDR) technique [23]. A three-rod (1.5 mm in diameter, 15 cm long) TDR stainless steel probe was vertically inserted into the upper tube. The electromagnetic capacitance and reflectance patterns of the growth mix were measured with a Tektronix 1502C cable tester (Tektronix, Beaverton, OR, USA). Ten growth mix samples of volumetric water content, from 10 to 100%, were prepared. A standard curve was regressed from the TDR measurement values of these samples and their volumetric water content ( $R^2 = 0.98$ ). The volumetric water content of the growth mix was calculated from the measured TDR values in the study and the standard curve.

In the first experiment (Study 1), the water volume needed to saturate soil in the upper tube (part-a) was determined. About two-thirds of this amount of deuterium-enriched water (1‰ concentration, 1 mL aliquots of 99.9 atom% $D_2O$  (Sigma-Aldrich, St. Louis, MS, USA) diluted in 1 L of tap  $H_2O$ ) was added to soil surface of the upper tube (part-a), as this amount of water would not permeate into the bottom tube. The  $\delta D$  value of the deuterium-enriched water was 4960‰, while the  $\delta D$  value of tap water which served as control was  $-220$ ‰. In the second experiment (Study 2), one-year-old dormant balsam poplar (*Populus balsamifera*) seedlings were grown in pots for two months before being

transferred to the upper tube of the split-root growth setup alongside the grasses. The root systems with the soil plugs of poplar seedlings were approximately 10 cm long. Space was made in the soil at the edge of the upper tube and the roots were positioned there. Two weeks later, when the poplar seedlings were successfully established in the upper PVC tubes and the newly grown roots had not reached the lower tube, the lower tube (part-b) was placed in 2 L of deuterium-enriched water for six days and no water was added to the upper tube (part-a). The soil volumetric water content of the lower tube (part-b) in Study 1 and upper tube (part-a) in Study 2 were continuously measured by TDR for six days at 6:30 and 21:30 each day (30 min after and before the dark period, respectively).

### 2.3. Hydrogen Isotope Analysis

In Study 1, on day 6, after adding water only to the upper tube segment, soil and leaf samples of three randomly selected plants were collected for hydrogen isotope analysis. Soil samples from the middle parts of both the upper and lower PVC tubes were collected and sieved through the 6-mesh sieve. In Study 2, when the poplar seedlings started showing signs of leaf wilting, the seedlings were harvested and the leaves were collected. The leaves of the slender wheatgrass and yellow sweet clover were harvested six days after the lower tube had been placed in deuterium-enriched water, and the soil from the upper tube was collected and filtered. After harvesting, leaf and soil samples were stored in  $-20\text{ }^{\circ}\text{C}$  freezer. For both leaf and soil samples, water was extracted using the cryogenic vacuum distillation method [24]. The hydrogen isotope analysis was performed with the Isotope Ratio Mass Spectrometer (IRMS) Delta Plus XP (Thermo, Dreieich, Germany), interfaced with a GasBench II (Thermo Fisher Scientific, Waltham, MA, USA) in G.G. Hatch Stable Isotope Laboratory in University of Ottawa. Hydrogen isotopic (i.e., deuterium) composition is expressed as:

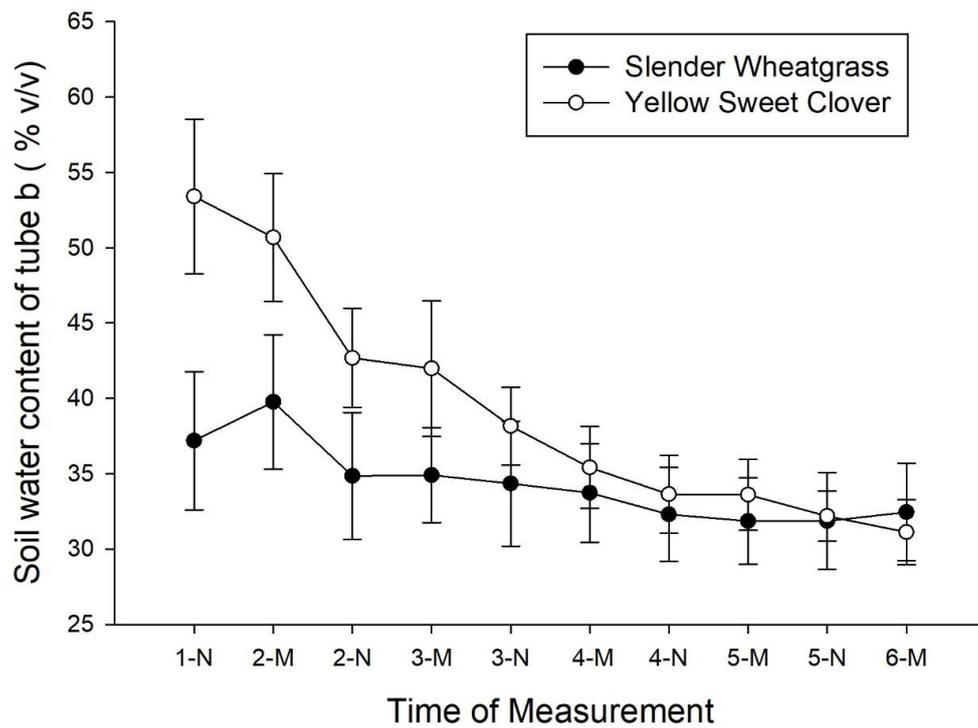
$$\delta\text{D} = \left( \frac{R_{\text{sample}}}{R_{\text{Standard}}} - 1 \right) \times 1000\text{‰} \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{Standard}}$  are the molar ratios of D/H of the sample and the standard Mean Ocean Water (i.e., V-SMOW), respectively [25].

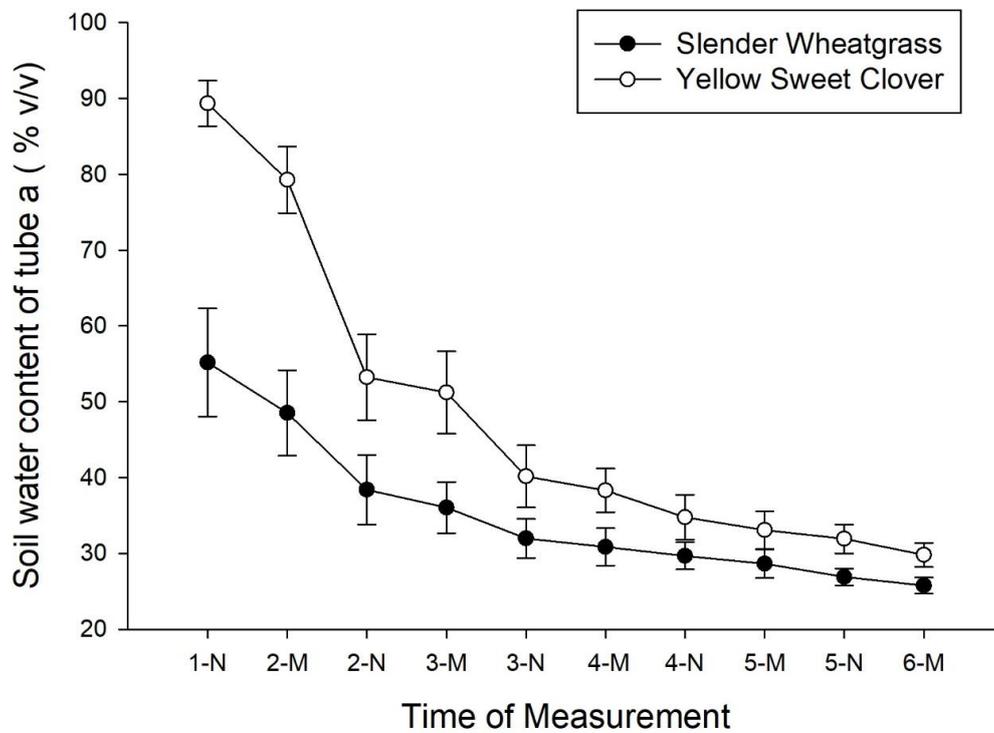
### 3. Results

In both studies, we did not observe an increase of volumetric water content in the soil of the tubes to which water had not been added (soil-b in Study 1, soil-a in Study 2) in the early morning right after the lights were turned on (Figures 2 and 3). The water content of non-watered soil in both studies became relatively stable starting on day 4 (Figures 2 and 3), which indicates that the roots were unable to absorb water from the soil.

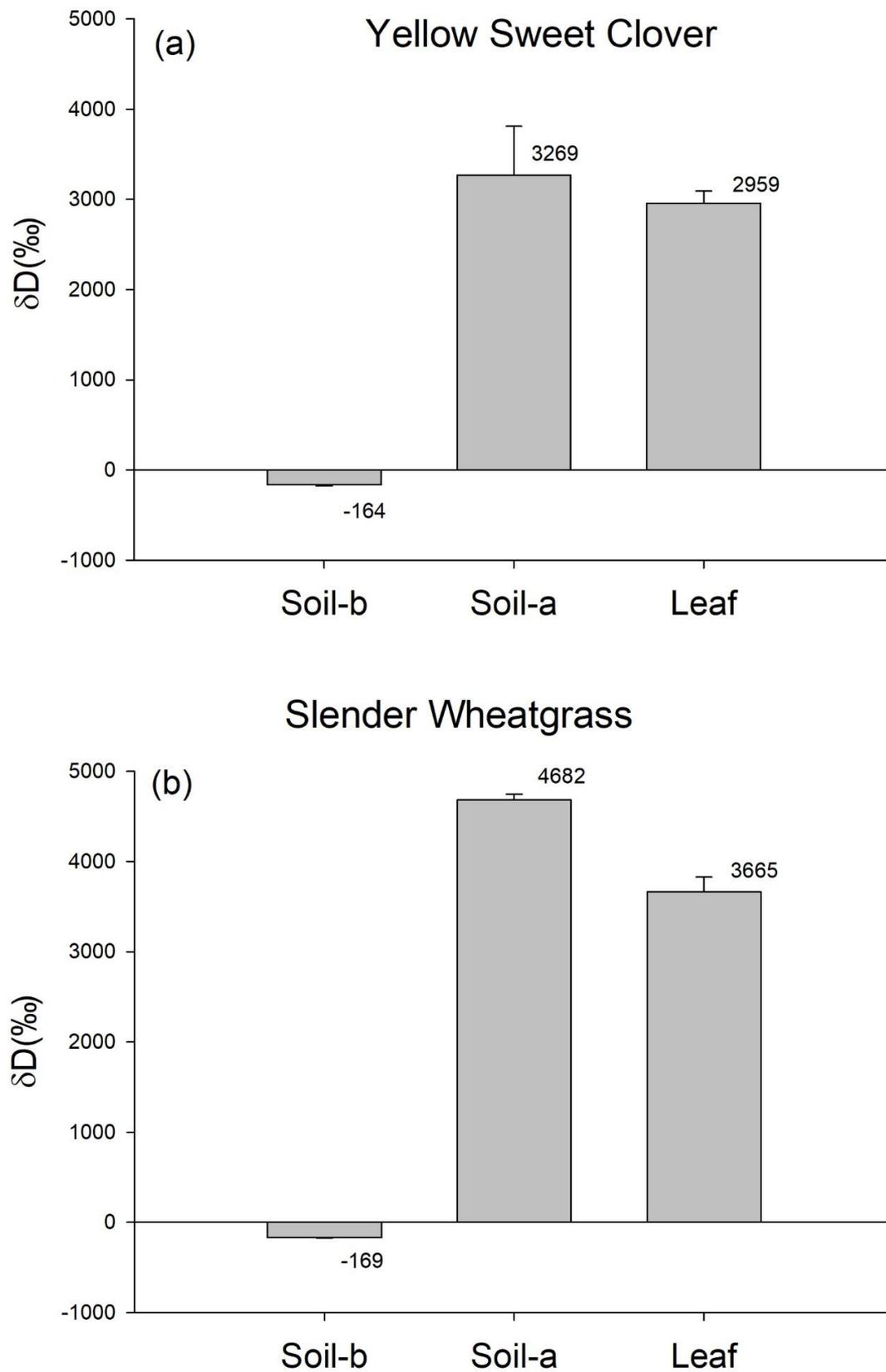
In Study 1, the  $\delta\text{D}$  of soil-b for clover and wheatgrass were  $-164\text{‰}$  and  $-169\text{‰}$ , respectively (Figure 4). In Study 2, the  $\delta\text{D}$  values of poplar leaves of seedlings grown together with clover and wheatgrass were  $-109\text{‰}$  and  $-119\text{‰}$ , respectively, and the  $\delta\text{D}$  values of soil-a for clover and wheatgrass were  $734\text{‰}$  and  $415\text{‰}$ , respectively (Figure 5).



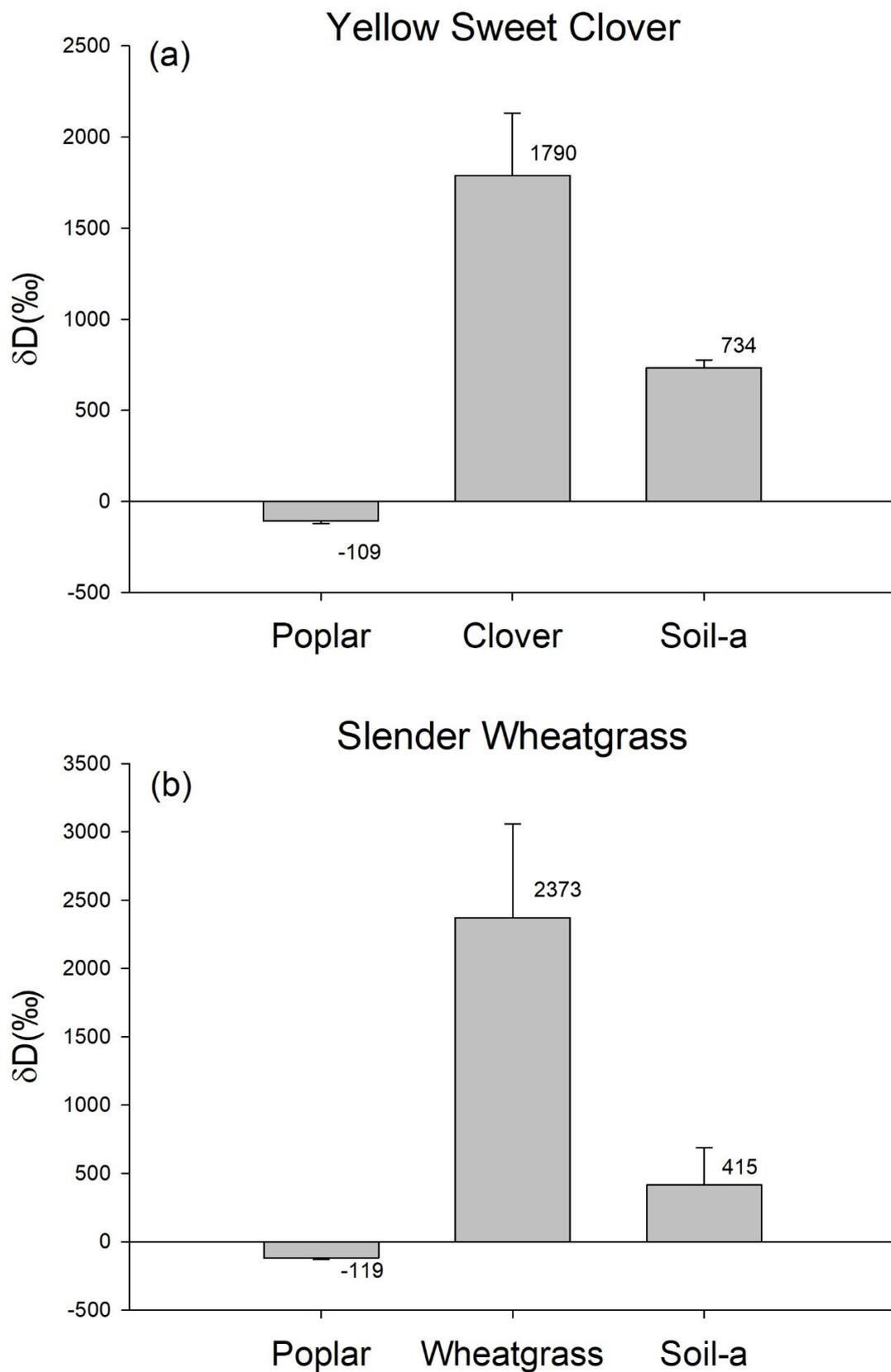
**Figure 2.** Diurnal patterns of soil volumetric water content in the lower PVC tube (part-b) when water was supplied only to the upper tube (part-a) in Study 1. Measurements were conducted at 6:30 in the morning (M) and at 21:30 at night (N). Means  $\pm$  SE (standard error) ( $n = 5$ ) are shown. *v/v* means volume to volume.



**Figure 3.** Diurnal patterns of soil volumetric water content in the upper PVC tube (part a) when the lower tube (part b) was placed in 2 L of the deuterium-enriched water in Study 2. Measurements were conducted at 6:30 in the morning (M) and at 21:30 at night (N). Means  $\pm$  SE ( $n = 5$ ) are shown.



**Figure 4.** Hydrogen isotope ratios ( $\delta D$ ) of extracted water from the soil in the upper tube (soil-a), lower tube (soil-b), and leaves of the yellow sweet clover (a) and slender wheatgrass (b) in Study 1, when deuterium-enriched water was supplied only to soil in the upper tube. The  $\delta D$  of the control water was  $-220\text{‰}$ . Means  $\pm$  SE ( $n = 3$ ) are shown.



**Figure 5.** Hydrogen isotope ratios ( $\delta D$ ) of the extracted water from soil in the upper tube (soil-a), poplar leaves, and leaves of the yellow sweet clover (a) and slender wheatgrass (b) in Study 2, when the lower tube (tube-b) was placed in the deuterium-enriched water. The  $\delta D$  of control water was  $-220\text{‰}$ . Means  $\pm$  SE ( $n = 3$ ) are shown.

#### 4. Discussion

Since the  $\delta D$  of tap water was  $-220\text{‰}$ , the  $\delta D$  values of soil-b in Study 1 and soil-a in Study 2 demonstrated that deuterium-enriched water was released from roots in the soil of the tubes where water was not added. However, hydraulically redistributed water was not detectable with the TDR technique, since the amount of redistributed water was relatively small and not enough to induce marked soil water content increase (Figures 2 and 3) [26]. Since the  $\delta D$  values in soil-a in Study 2 were greater compared with soil-b in Study 1, the potential for upward HR in both species is greater than for downward HR. The much higher  $\delta D$  values of water extracted from poplar leaves than that of tap water evidenced that deuterium-enriched water absorbed from the lower compartment (part-b) by poplar roots was released to the upper compartment (part-a) in both clover and wheatgrass. In Study 1, the  $\delta D$  values in the soil of the upper tube (part-a) and leaves of the wheatgrass and clover were similar, which was expected indicating that roots absorbed water mostly from the upper tube (Figure 4). In study 2, although the  $\delta D$  values of the clover leaves were lower than in the wheatgrass leaves, the  $\delta D$  of soil in the upper tube (part-a) with clover was higher than that with wheatgrass (Figure 5). This suggests that the upward HR potential of clover was stronger than that of wheatgrass.

Over the short treatment duration in Study 2, there was no measurable shoot or root biomass growth in poplar seedlings. Poplar competed for water uptake with slender wheatgrass and yellow sweet clover in the upper tube and also absorbed water discharged by HR from these two herbaceous plants. It appeared that the competition for water played a major role in the responses of poplar seedlings since they showed the signs of wilting soon after the onset of the deuterium-enriched water treatment of the lower tube. Therefore, the HR of slender wheatgrass and yellow sweet clover did not appear to prevent the effects of drought stress in balsam poplar. A number of studies have argued that the positive effect of water supply by HR is overwhelmed by soil water competition between the donor and receiver plants [9,27]. Soil texture can affect the amount of water that roots can exude and in coarser soil types, HR capability of root systems was reported to decrease [1]. The growth mix used in this study contained large amounts of peat moss (coarse texture), which might lower the HR potential of both the examined herbaceous plants. Since in Study 2, the roots of herbaceous plants and poplar seedlings had limited space, the competition for water resources between the two plants might be sufficiently significant to contribute to rapid wilting of poplar seedlings. Sekiya et al. [13] reported that forage plants with sheared shoots could supply large amounts of water to associated crops and enhance their growth. Such an enhancement of HR potential in agronomic grasses on reclamation sites would be highly desirable in facilitating the establishment and growth of planted tree seedlings.

#### 5. Conclusions

The hydrogen isotope analysis demonstrated the upward and downward HR by slender wheatgrass and yellow sweet clover. However, in this study, the amount of water released by the roots of these plants was probably too small and did not appear to alleviate drought stress in associated balsam poplar seedlings. However, even a small amount of exuded water by HR could play a crucial role in maintaining the rhizosphere microbial population and mycorrhizal associations that could increase water and nutrient uptake in plants under drought conditions [7]. Therefore, more studies should be conducted to investigate the benefits of HR on mycorrhizal associations in plants and the possibility of HR enhancement by shoot shearing, and possibly other techniques, to increase the amount of water released, especially under prolonged summer drought conditions.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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