# A Novel Technique for Rapidly Separating Willow Roots from Clay Soil

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## Abstract

Numerous studies have examined the root dynamics of willow biomass energy crops growing on medium to coarse-textured soils, using either soil coring, minirhizotron techniques, or a combination thereof. However, neither approach is well suited for studying roots in soil of high clay content. Our objective was to test the efficacy of using a simple baking soda ($NaHCO_3$) pre-treatment for facilitating the separation of willow roots from a Vertisol (70% clay). Soil cores were collected from within a willow variety trial plot of Tully Champion (*Salix viminalis* x *S. miyabeana*) and were either conventionally washed (i.e., no pre-treatment) or washed following a pre-treatment consisting of shaking the sample for 15 min with either deionized water or 1.2*M* $NaHCO_3$. Measurement variables included washing duration, water usage, and recovery of fine (< 2 mm) and coarse roots. The ranking of washing duration and water usage was 1.2*M* $NaHCO_3$ pre-treatment < deionized water pre-treatment < conventional washing. Compared to conventional washing, the 1.2*M* $NaHCO_3$ pre-treatment reduced the washing duration and water usage by 45 and 61%, respectively, while increasing the fine-root recovery by 29%. There was no significant difference ($P > 0.05$) in coarse root recovery among the three washing methods. Developing a quicker technique of separating willow roots from high clay content soilshould promote further investigations of root growth dynamics within this traditionally difficult soil type.

## Introduction

Although initially proposed as a renewable dedicated bioenergy feedstock, with indirect environmental benefits through fossil fuel displacement (Christersson et al. 1993), purpose-grown shrub willow (*Salix* spp.) are increasingly employed in an array of ecotechnology applications that directly benefit the environment (Mirck et al. 2005; Kuzovkina and Volk 2009). As with all plant species, willow depends on its root system to support its successful establishment and growth. Furthermore, understanding willow root growth dynamics is particularly important considering its coppice regeneration and concomitant dependency on its perennial root stock to provide stability for the above-ground growth, and water and nutrient uptake (Volk 2001). Notwithstanding the apparent need for reliable root biomass data, examining roots, particularly the fine-root fraction, is tedious, labour intensive, destructive, and expensive given the inaccessibility of root systems compared to the above-ground biomass component. Soil coring provides the most accurate estimate of below-ground biomass partitioning of willow growing on high clay content soil. However, separating willow roots from the clay matrix requires considerably more time and water compared to loam or sandy soil (Rytter 2001), which also increases the probability of fine root damage and loss (Stadnyk 2010). Such logistical difficulties may explain the scarcity of studies reporting willow root growth dynamics in high clay content soil and point toward a need for developing a better alternative to conventional washing for root separation from the clay soil.

Solonetzic or sodic soils occur worldwide and their genesis results from the presence of abundant sodium (Na)-salts within the soil profile; either inherent within the parent material or supplied by groundwater discharge (Miller and Brierley 2011). The high exchangeable-Na content causes soil alkalization, with the Na-saturated clay minerals having thicker diffuse double layers, causing repulsion and deflocculation of clay particles, which eluviate from the A to the B horizon to form a dense hardpan layer (Pawluk 1982). The objective of this study was to apply these first principles of Na-induced dispersion of soil colloids to develop an improved method of separating willow roots from high clay content soil by using a $NaHCO_3$ pre-treatment before washing. We hypothesized that shaking soil core samples in a solution with abundant Na, would saturate the clay surfaces with Na, disperse clay aggregates and liberate the bound roots (especially the fine roots), resulting in more efficient root-soil separation and increased fine-root recovery compared to conventional washing. Developing a technique that rapidly separates willow roots from clay-rich soil will not only save time, but also conserve water, which is advantageous for supporting global initiatives targeting the sustainability of this vital resource (Holger 2009). Additionally, improving fine root estimates within clay soils should facilitate further root studies involving this traditionally difficult soil type.

## Materials and Methods

### *Study Site and Willow Variety*

The samples for this study were collected in the fall of 2011 from a five-year-old shrub willow variety trial located on the University of Saskatchewan campus in Saskatoon, Saskatchewan, Canada (UTM coordinates: 13U 389970 5776342). The site information is described in detail in Hangs et al. (2011b) and a brief summary follows. The soil is a heavy clay Orthic Vertisol (Sutherland Association; 70% clay content), developed on glacial lacustrine parent material, with a pH and electrical conductivity (dS/m) of 7.1 and 0.33, respectively. The semi-arid temperate location receives on average 350 mm of annual precipitation (70% occurring from May to September) and has a mean annual temperature of 2°C, with approximately 112 frost-free days. The Agriculture Capability Classification rating of the soil is Class 2, with moderately severe limitations due to a lack of precipitation (SCSR 1978). In 2007, thirty willow varieties, developed by the SUNY-ESF breeding program, were planted as follows: each varietal plot (6.3 x 7.8 m) consists of 78 plants (three double-rows of 13 plants/row), with spacings of 1.5 m between the double-rows, 60 cm between rows within the double-row, and 60 cm between plants within the row; resulting in a planting density of approximately 15,873 plants/ha (Fig. 1). The above-ground willow biomass within all variety plots was harvested in the spring of 2011, so at the time of this study, each multi-stemmed willow plant (i.e., stool) consisted of one-year-old stems on five-year-old roots. The willow variety used in this study was Tully Champion (*Salix viminalis* x *S. miyabeana*); selected given its superior above- and below-ground biomass production on both arable and marginal soils (Smart and Cameron 2008; Hangs et al. 2011a).

### *Soil Core Collection and Washing Procedures*

Thirty-six soil cores were systematically collected from within a single plot of Tully Champion (Fig. 1), using an 8 cm diameter bucket auger, and sampled down to a depth of 30 cm, where the majority of willow roots occur (Rytter 1999; Heinsoo et al. 2009). The soil cores were randomly divided into three groups and assigned to one of three treatments prior to washing: i) conventional, ii) shaken in deionized water for 15 min, or iii) shaken in 1.2$M$ $NaHCO_3$ for 15 min. A 1.2$M$ $NaHCO_3$ solution was prepared by dissolving a standard 500 g box of Arm and Hammer® (Princeton, NJ, USA) baking soda into 5 L of deionized water. Each soil core was placed in a 11.3 L Rubbermaid® storage container, submerged in either deionized water or 1.2$M$ $NaHCO_3$, and shaken for 15 min at 144 rpm on a G10 Gyratory Shaker (New Brunswick Scientific, Edison, NJ). All soil core samples were manually washed with tap water by a single person and the roots were collected using a double-sieve system (2 and 0.5 mm mesh). The roots were divided into fine (< 2 mm) and coarse-size fractions, dried (40 $^{o}$C) to a constant weight, and weighed. The non-crop vegetation within the plot was sparse, due to extensive vegetation management and canopy closure (2011b), thus all roots were assumed to be that of willow. The time required to wash each soil core and the amount of water used (Water Saver™, AbsolutelyNew, Inc. San Francisco, CA) were both measured. The water usage data included the volume of deionized water used in the two shaking pre-treatments; however, neither the pre-treatment setup period nor the 15 min shaking time were included in the washing duration measurement.

### *Relationship Between Above-ground Biomass and Recovered Root Biomass Fractions*

A conventional allometric equation was developed to estimate above-ground willow biomass, by calibrating measured stem diameter (at 30 cm height) with harvested leafless biomass from 12 stems representing the diameter range within the plot (Arevalo et al. 2007). The allometric equation was derived using a simple non-linear power regression expressed in the following:

$$HB = aD_{30}{}^{b},$$

where $D_{30}$ and HB are the measured stem diameter and harvested oven-dry biomass (stem + branches) and a and b are the allometric coefficient and exponent constants, respectively. The stems of each willow stool within the plot were likewise measured, their diameters applied to the allometric equation ($y = 0.0847x^{2.645}$; $r^2 = 0.99$; $P < 0.001$; n = 12), and summed to estimate above-ground willow biomass for each stool. In order to examine the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass after each washing treatment, the average biomass of the four stools surrounding each collected soil core was compared to the recovered fine and coarse root biomass of the core (Fig. 1).

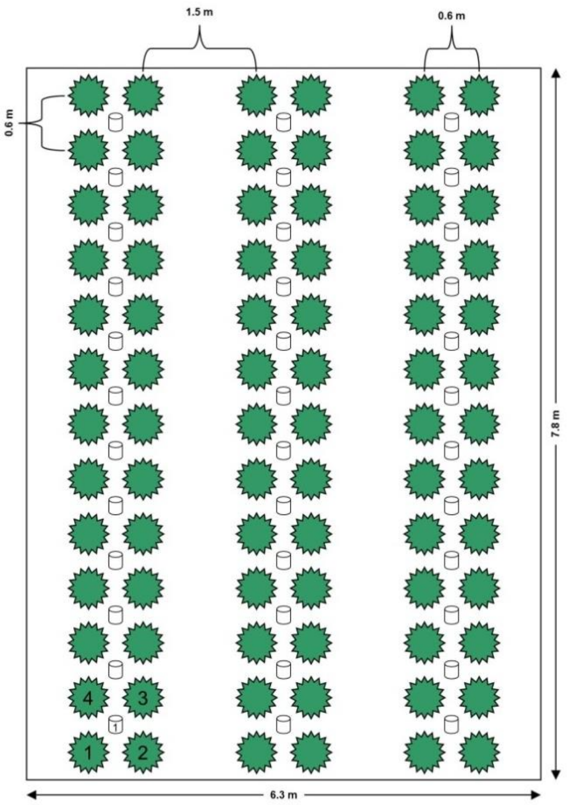

**Figure 1.** Willow variety trial plot layout and soil core sampling locations. Note: in order to examine the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass after each washing treatment, the average stool biomass (e.g., 1, 2, 3, and 4) surrounding each collected soil core (e.g., 1) was compared to the recovered fine and coarse root biomass of the core.

### *Statistical Analyses*

Measurement variables were analysed using PROC MIXED in SAS (version 9.2; SAS Institute Inc., Cary, NC., USA). Means comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05, with groupings obtained using the pdmix800 SAS macro (Saxton 1998). PROC REG was used to carry out simple linear regressions with pooled data (n = 12) to quantify the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass after each washing treatment. Normality of distributions (PROC UNIVARIATE) and homogeneity of variances (Bartlett's test) of all data sets were checked prior to the analysis. No data transformations were necessary.

## Results and Discussion

### *Washing Duration and Water Usage*

The time required to separate the willow roots from the heavy clay soil sample ranged from 6.9 to 12.4 minutes per core among the treatments (Fig. 2a). Shaking the soil cores in solution for 15 min, in either deionized water or 1.2*M* $NaHCO_3$ prior to washing, reduced the

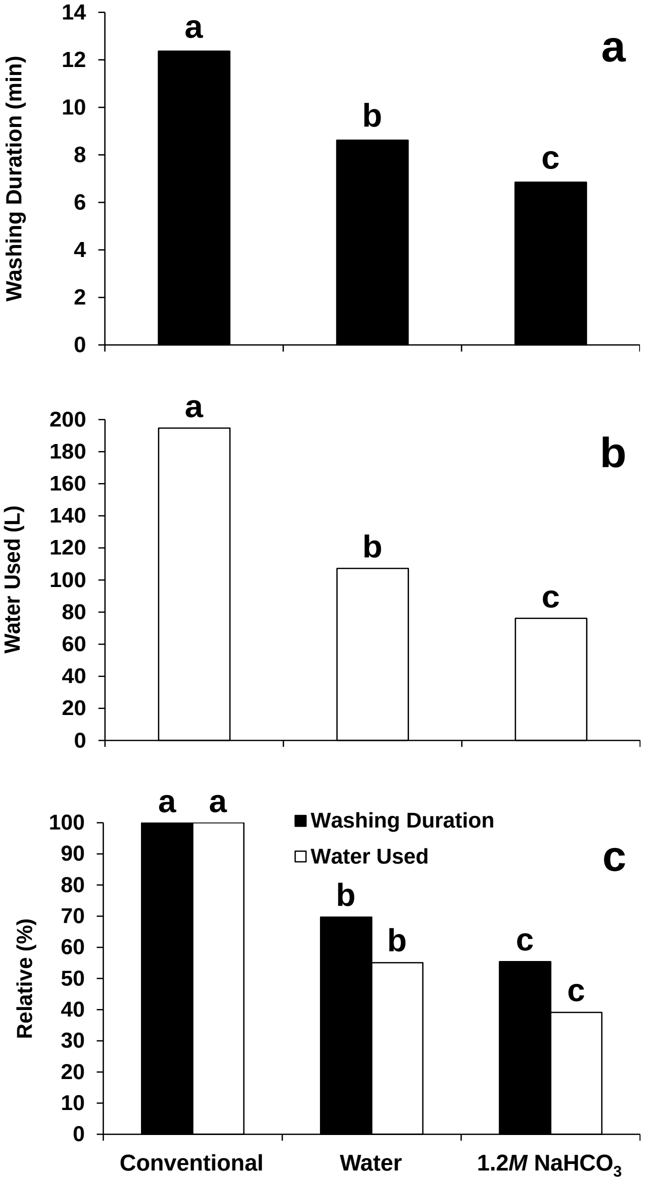

**Figure 2.** Mean (n = 12) washing duration (a) and water used (b) to separate willow roots from a heavy clay soil core, either conventionally washed or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water or 1.2*M* $NaHCO_3$. Relative differences among the methods are also shown (c). Note: Treatment bars having the same letter are not significantly different ($P > 0.05$) using LSD. For (c), only means comparisons within measurement variable are valid.

washing duration compared to conventional washing. The amount of water used per core sample varied from 76.1 to 194.7 L among the treatments (Fig. 2b). The most efficient use of time and water occurred following the 1.2*M* $NaHCO_3$ pre-treatment, which reduced washing duration and water usage by 45 and 61%, respectively, compared to conventional washing (Fig. 2c). The measured differences in washing duration and water usage among the treatments are elucidated by visually comparing the soil core samples prior to washing. Specifically, unlike conventional washing, where the retention of subangular blocky aggregate structure is apparent (Fig. 3a), shaking the core sample in deionized water dissolved some aggregates (Fig. 3b), due to the destabilizing slaking effect of water immersion (Hillel 1982), which then facilitated the separation of roots from the heavy clay soil matrix. Shaking the soil core with 1.2*M* $NaHCO_3$, however, completely dispersed the clay particles, destroying aggregates that resulted in a suspension that was more conducive for root separation (Fig. 3c). The abundant exchangeable-Na exchanged with the $Ca^{2+}$ on the clay mineral surfaces, deflocculating the soil aggregates, while the free $HCO_3^-$ reacts with liberated $Ca^{2+}$ to form calcium bicarbonate, which commonly occurs within alkaline calcareous sodic soils (Pawluk 1982), thus removing an important flocculating element from solution. No attempt was made to examine the effects of different concentrations of $NaHCO_3$ and/or shaking interval on subsequent washing duration and water usage. Both parameters may need adjusting to account for differences in the % clay of the soil core. However, the $NaHCO_3$ concentration of 1.2*M* used in this study is close to the saturation limit of $NaHCO_3$ at room temperature, therefore, it is expected that increasing the shaking time would be the best approach to pre-treat soil cores with clay contents in excess of 70%, followed by replenishing the pre-treatment solution with fresh 1.2*M* $NaHCO_3$.

### *Recovery of Fine and Coarse Roots*

The fine root biomass estimates among the treatments ranged from 2.6 to 3.3 Mg/ha (Fig. 4a). Shaking the soil cores in 1.2*M* $NaHCO_3$ increased fine root recovery by 29% compared to conventional washing (Fig. 4c). There were no significant differences ($P > 0.05$) in coarse root recovery (Fig. 4b) or fine root:coarse root (data not shown) among the treatments. Willow fine roots are more susceptible to damage and loss during washing relative to coarse roots (Stadnyk 2010); consequently, the measured increase in fine root biomass recovery after shaking in 1.2*M* $NaHCO_3$ is not surprising, given the effectiveness of this pre-treatment in separating the roots from the high clay content soil. Minimizing the washing duration undoubtedly supported a greater recovery of fine roots, especially the smaller higher-order fine root fraction (i.e., < 0.5 mm) that can comprise the majority of willow fine roots (Rytter and Rytter 1998). Although not quantified, visually there were considerably smaller fine roots recovered after the 1.2*M* $NaHCO_3$ pre-treatment compared to the other two treatments. Reduced washing activity not only creates less disturbance (i.e., fragmentation) of the root system that will increase the likelihood of collecting intact root branches in a 0.5 mm sieve, but also presumably decreases leaching losses of water-soluble compounds from root tissues (Puttsepp et al. 2007).

### *Relationship Between Above-ground Biomass and Recovered Root Biomass Fractions*

The measured above-ground willow biomass was only correlated with the fine root biomass estimates after the 1.2*M* $NaHCO_3$ pre-treatment, albeit the correlation was weak ($r^2 = 0.34$; Fig. 5). Removal of the seemingly errant fine root observation of 4.1 Mg $ha^{-1}$ (Fig. 5c) from the regression analysis greatly improves the model (i.e., $r^2 = 0.71$, $P < 0.004$); however, a Grubbs' test (alpha = 0.05) failed to identify it as an outlier and there was no visually apparent reason for the atypical fine root proliferation at that location to justify its removal from the data set. Notwithstanding the model's relatively poor goodness of fit, the 1.2*M* $NaHCO_3$ pre-treatment does appear to provide fine root biomass data that is biologically more meaningful, compared to the other two treatments, which may be a function of its ability to increase the

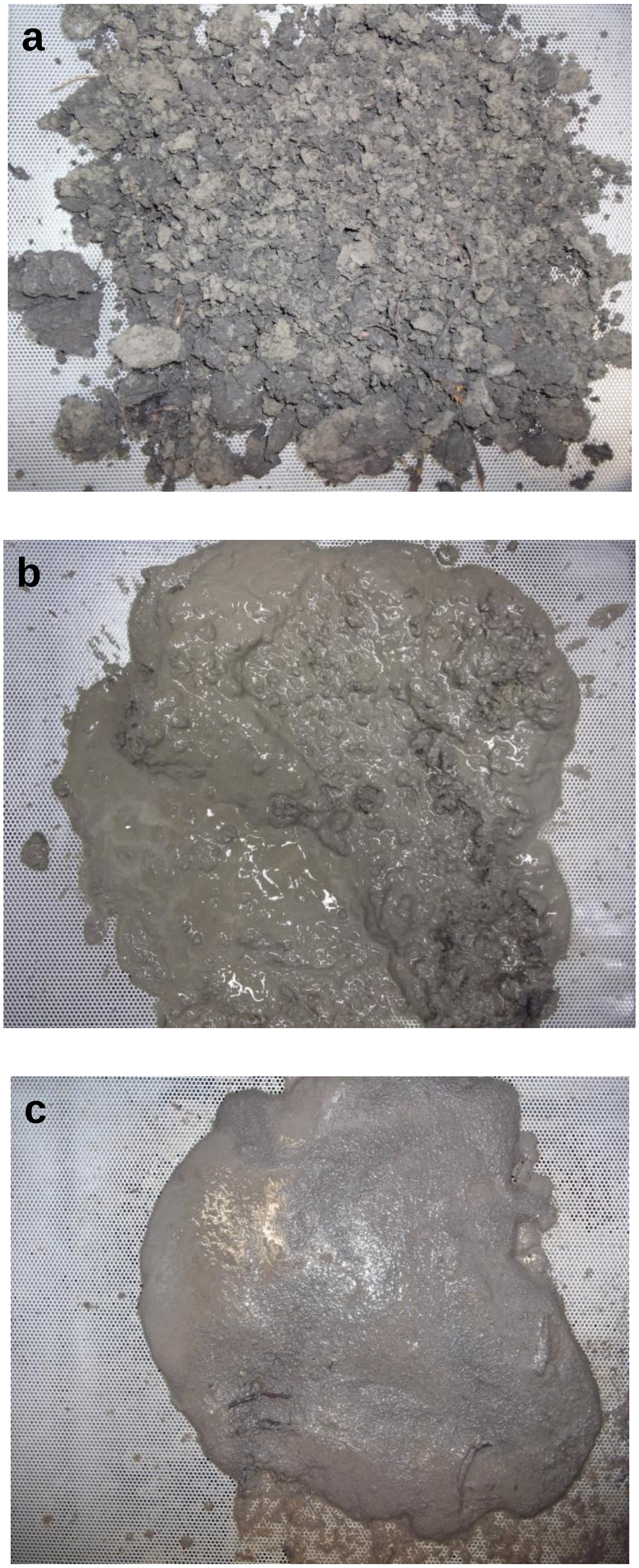

**Figure 3.** Heavy clay soil cores prior to manual washing without pre-treatment (a) or shaken in solution for 15 min with either deionized water (b) or 1.2*M* $NaHCO_3$ (c).

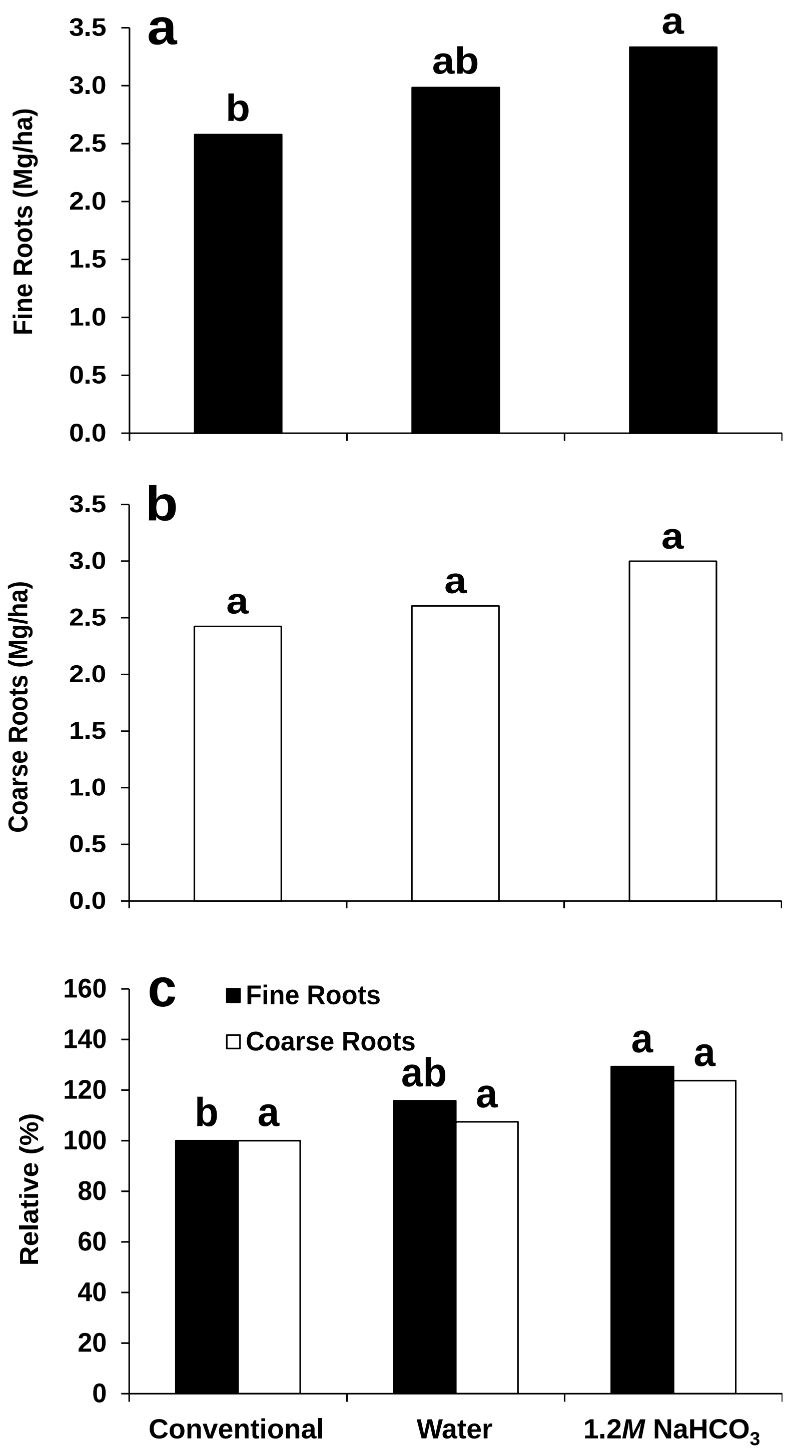

**Figure 4.** Mean (n = 12) willow fine (< 2 mm; a) and coarse root (b) biomass recovered from heavy clay soil cores, either conventionally washed or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water or 1.2*M* $NaHCO_3$. Relative differences in willow root recovery among the methods are also shown (c). Note: Bars having the same letter are not significantly different ($P > 0.05$) using LSD. For (c), only means comparisons within a root size fraction are valid.

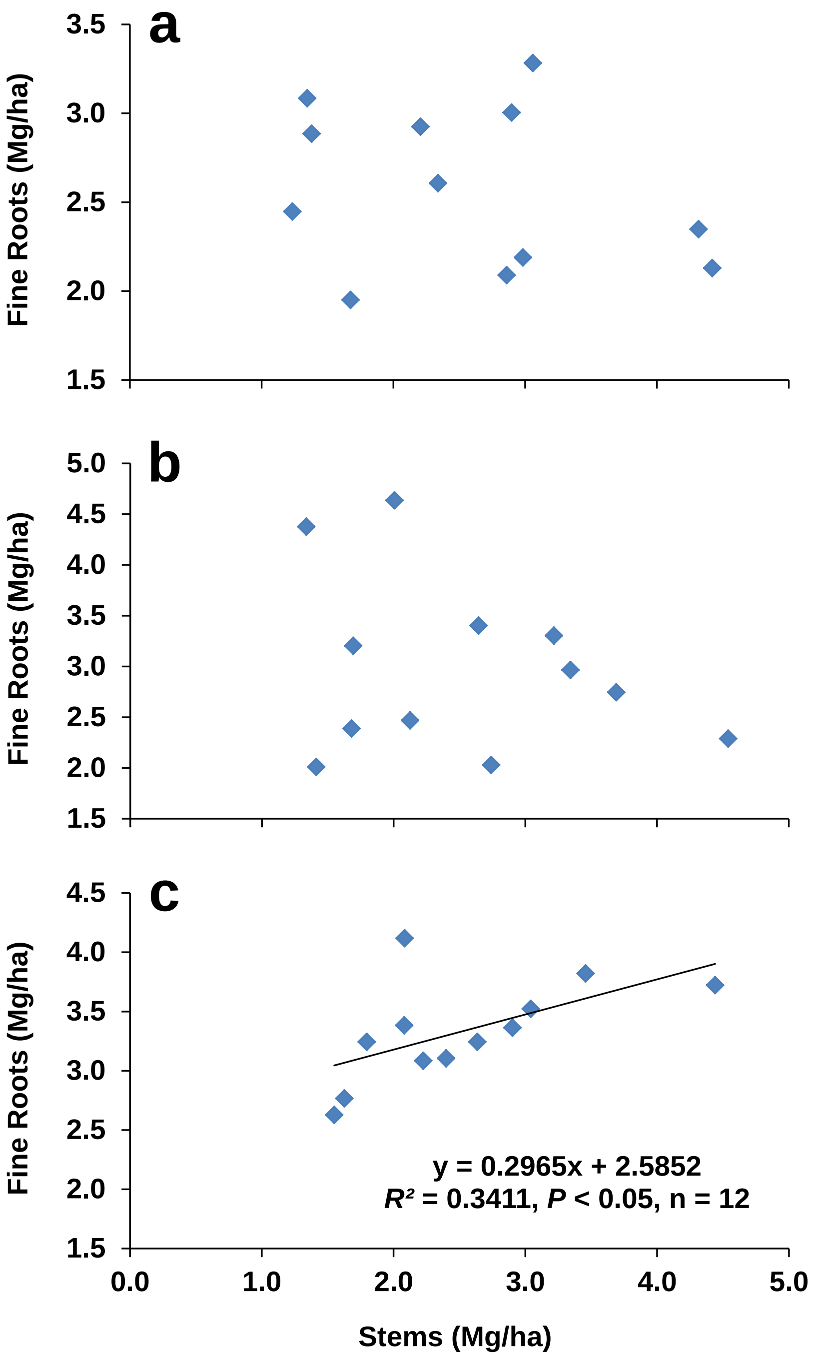

**Figure 5.** Relationship between harvested oven-dry willow biomass (stem + branches) and fine root biomass recovered from heavy clay soil cores, either conventionally washed (a) or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water (b) or 1.2*M* $NaHCO_3$ (c).

recover of smaller higher-order fine roots. The lack of correlation between above-ground willow biomass and coarse root biomass estimates, regardless of soil core treatment, supports the critical role fine roots play in water and nutrient uptake for supporting willow productivity (Rytter and Hansson 1996). This will be particularly important in the semi-arid climate of Saskatchewan, where moisture availability often is considered the primary controller limiting growth for both annual and perennial plant species (Akinremi et al. 1996; Hogg and Schwarz 1997).

## Conclusion

Roots are an integral component of plant morphology and physiology, in addition to an important component of the plant carbon sink. Therefore, documenting root growth dynamics, in particular the fine root fraction, is essential. Minirhizotrons are the preferred method of studying willow roots, but are unsuitable for use within high clay content soil. In these clay soils, conventional soil cores will provide the most reliable estimate of below-ground biomass partitioning of willow, but separating the roots from the clay is difficult with conventional washing. A pre-treatment involving shaking soil core samples with 1.2$M$ $NaHCO_3$ for 15 min prior to washing decreased the amount of time and water required to separate willow roots from the clay matrix, while increasing fine-root recovery. The results of this study should facilitate and promote more investigations of root growth dynamics in high clay content soils, which may otherwise be avoided due to the inherent difficulties in root separation using conventional washing techniques. Further studies will advance our understanding of willow root growth dynamics, along with attendant management implications, when cultivating this fast-growing woody species on high clay content soils.

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