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# Total and available soil carbon fractions under the perennial grass Cynodon dactylon (L.) Pers and the bioenergy crop Arundo donax L.

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#### ABSTRACT

Understanding and quantifying the impact of bioenergy crops on soil carbon (C) storage is an essential component of crop management. Our objectives were to (i) compare total (TC), organic (OC), and inorganic carbon (IC) storage under Cynodon dactylon (L.) Pers and the energy crop Arundo donax L. along the soil profile, and (ii) determine the effect of these crops on available soil C (measured as hot water extractable C, HC) as an indirect indicator of soil C changes. The study site was within the Rio Grande floodplain in Quemado, Texas covered by A. donax and C. dactylon. Soil samples were taken from five soil depths: 0-10, 10-20, 20-30, 30-40, and 40-50 cm at 125 locations in a 34.5 ha field; TC, IC, and HC were measured and OC was derived. In all four C pools, soils under A. donax had higher C content (volumetric C or Cv, kg m<sup>-2</sup>) than soils under C. dactylon, except for IC at the top two depths. Larger soil C storage under A. donax as compared to C. dactylon was consistent throughout the profile. The effect was most pronounced for volumetric HC content (HCv) with 43% higher amount under A. donax than C. dactylon at 0-10 cm depth. In areas, where A. donax is considered an invasive species, the available biomass can be used for bioenergy production and the higher soil carbon under A. donax can provide additional economic return in a C economy.

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#### 1. Introduction

Efforts to increase soil carbon (C) storage through conservation management have gained momentum in the last few decades, particularly to counter the effects of global warming. Soil C has been a key component of land management for a long time, as it is important for nutrient availability, moisture holding capacity, and soil health as well as several

Abbreviations: OC, organic carbon; TC, total carbon; IC, inorganic carbon; HC, hot water extractable carbon; Cc, carbon concentration in g kg<sup>-1</sup> of soil; Cv, volumetric carbon or carbon stock in kg m<sup>-2</sup>.

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ecosystem functions of soil such as filtration of water and contaminants. Therefore, alternative management practices that can enhance soil C sequestration have attracted significant research attention [1-3]. Bioenergy has attracted increasing research and policy support aiming to reduce green house gas emissions and the dependence on fossil fuels. Currently, about 4% of the total energy consumption in the U.S. is derived from biomass energy [4] and it is estimated that up to one third of the transportation fuels can be replaced by biomass energy in the US [5]. Many studies using life cycle assessment technique have reported that biofuels reduced total fossil fuels consumption [6-10]. For example, Schmer et al. [7] reported that switchgrass produced 540% more renewable fuel as compared to nonrenewable fuel used in the process. However, there are various environmental concerns associated with different sources of bioenergy. For example, grain ethanol production is well established in terms of technology and industrial infrastructure, but it is not considered sustainable because it diverts food grains from food and animal feedstock needs. Hill et al. [10] reported that replacing petroleum with either ethanol or biodiesel (from food crops) was not possible without impacting food supplies. Additionally, the energy spent in growing these food grains incurs C cost, while the agronomic chemicals and tillage place further demands on the environment [11,12]. Cellulosic ethanol from crop residues is considered to be a more sustainable alternative for bioenergy production since they do not require additional agronomic inputs. However, crop residues, when left in the field, perform important ecological functions such as erosion control, improvement of soil physical properties and maintenance of soil C levels. Thus, removal of crop residues poses enormous risks for preserving soil health. For example, Anderson-Teixeira et al. [13] reported that removal of as little as 25% corn residues resulted in reduction of soil C stocks. They found that even though perennial grasses accumulated soil C, a period of C payback time was required to restore the soil C lost due to cultivation (e.g., a century for sugarcane). Intensively managed perennial grasses and wood crops are also reported to incur higher C costs due to fossil fuels consumed directly or indirectly during cultivation. For example, Pimentel and Patzek [14] reported that many of the biofuel sources such as corn, soybean, sunflower, switchgrass, and wood biomass actually required 29, 27, 118, 50, and 57% more fossil fuel for production compared to the fossil fuel replaced by the biofuel produced from the feedstocks. Similarly, when other environmental impacts caused by increased tillage, use of chemical fertilizers and pesticides or reduction in biomass input to soil and resultant decrease in soil C and nutrients were considered, the cost outweighed the benefits in case of high input biomass feedstocks such as corn grains or conversion of native lands to cultivated biofuel crops [13]. As a result, in a review of life cycle analyses of bioenergy systems, Cherubini et al. [15] concluded that determination of the C cost of bioenergy is complex and dependent on multiple, highly variable factors. However, the authors also concluded that using waste biomass or crop residues and low input bioenergy crops that offer greater ecosystem services than the systems they replaced, e.g. reforestation of degraded lands, could offer more sustainable and carbon negative solutions for bioenergy.

Therefore, more research is needed for bioenergy feedstocks, which sequester C and require minimal additional inputs. For example, low input - high diversity grasslands and restored prairies have been reported to offer high amounts of bioenergy feedstock without adversely affecting the soil C stocks [16]. Arundo donax L. (Giant Reed) is such an excellent bioenergy feedstock with a gross heating value of 17.2 MJ  $kg^{-1}$ of dry leaf matter [17]. It is a fast growing plant and can reach up to 8–9 m height and up to 75 t  $ha^{-1}$  yield under optimum conditions [17,18]. It is capable of growing under dry conditions and without herbicides [17,19]. A. donax has been cultivated in parts of Europe, Africa, Asia, and the Middle East for thousands of years and has been present in the U.S. for more than a century [20]. Researchers have reported the suitability of A. donax feedstock for ethanol [21] and net positive energy output when managed for bioenergy production [22]. Angelini et al. [22] reported that when A. donax was fertilized, grown without irrigation, and harvested annually it had a mean energy yield of 627 GJ  $ha^{-1} y^{-1}$  over 12 years, whereas the mean energy yield for Miscanthus was only 467 GJ ha<sup>-1</sup> y<sup>-1</sup>. The energy input for both crops was 17 GJ t ha<sup>-1</sup> in the first year and 12.1 GJ t  $ha^{-1}$  every year from second year (average 12.5 GJ t  $ha^{-1}y^{-1}$ ). These energy yield studies suggest that use of the biomass of A. donax for bioenergy can be a sustainable alternative.

In the U.S., A. donax has been declared an invasive species in seven states, California, Nevada, Arizona, New Mexico, Texas, Georgia, and Virginia [23] and extensive efforts are expended for control and eradication of this species. Most of the eradication techniques recommended for this species, such as root excavation, mechanical removal, and herbicide treatment of the cut stems, require proper disposal of the A. donax debris. The decomposition of A. donax canes is slow; chipping requires heavy-duty equipment and C expenditure, while burning is restricted due to air quality considerations. In such cases using the removed biomass for bioenergy can ensure proper disposal of the debris and reduce the net cost of control measures while offering additional environmental benefits. In areas where control measures are not feasible, use of the available biomass for biofuel (as an intermediate measure) can also offer economic returns and reduce the fire hazard, since A. donax is highly inflammable. This can be particularly attractive in states such as California and Georgia where commercial cellulosic ethanol plants using waste biomass to produce electricity are already operational or are under construction. Moreover, A. donax is an environmental concern only when grown near waterways or in cases of improper disposal [24]. It has multiple uses including fiber, fodder, roofing material, and wind instruments, with existing commercial plantations in California for musical instruments [20]. Therefore, utilization for bioenergy as part of the control strategy of this species can be an ecologically and economically sound alternative.

However, it is necessary to determine the effect of these bioenergy sources on soil C storage. While extensive research has been conducted on soil C in grain ethanol crops [8,25] and cellulosic ethanol from food crops and their residues [16,26], soil C storage under *A. donax* requires further studies. It is also necessary to determine the effects of these crops not only on soil C stock, but also on labile C pools, which predict long-term changes in total C and perform important ecological functions such as providing energy source for soil organisms [27]. Bermuda grass or *Cynodon dactylon* (L.) Pers was used in this study for comparison, because it is used commonly in grazing lands, for turfgrass or soil cover as well as for hay and silage production in the tropical, subtropical and warm temperate regions worldwide [28]. In recent years, its potential for biomass energy production has also been reported [21,28]. In the southeastern US, it is the most commonly used forage grass. Hence, this study was undertaken with the objectives to (i) compare total, organic, and inorganic soil C storage under C. *dactylon* and the energy crop A. *donax* along the soil profile, and (ii) determine the effect of these crops on available soil C (as measured by hot water extraction, HC).

#### 2. Material and methods

#### 2.1. Site description

The study site was located near Quemado, Texas on a farmerowned floodplain near the Rio Grande River (28.9587 N, 100.6450 W). Climate data from the weather station Eagle Pass3n (30 km from Quemado, TX, Coop ID 412679) was used. The climate is subtropical, with 546 mm average annual rainfall, 21.5 °C mean annual temperature, 14.9 °C average annual minimum temperature, and 28.2 °C average annual maximum temperature [29]. The study area is mapped as Rio Grande soil series (coarse-silty, mixed, active, calcareous, hyperthermic Aridic Ustifluvents). These soils are very deep, well drained, moderately to rapidly permeable, and formed on alluvium derived from mixed sources. These floodplain soils are calcareous throughout to the surface. The land has been managed for grazing in the last 40 years. The farmer planted C. dactylon as part of a long-term management strategy to eradicate A. donax for the last 40 years, though large parts of the field are still under A. donax.

#### 2.2. Experimental design

The field was 34.5 ha in size with coverage of A. *donax* and C. *dactylon* in form of distinct patches. According to our interview

with the property owner, A. donax had been present for more than 40 years and C. dactylon was planted 30 years ago. A remote sensing image (Digital Ortho-photo Quarter Quadrangles aerial imagery from the Texas Natural Resource Information System) was used to determine the spatial distribution of the two plant species (A. donax and C. dactylon). Additionally, an apparent soil electrical conductivity (ECa) and elevation survey was performed [30]. Apparent electrical conductivity has been shown to improve the spatial characterization of soil organic C [31]. The role of topography in controlling soil C is also well known [32] and elevation has been used successfully to evaluate soil C in floodplains, similar to our study area [33]. Additionally, organic C in soils is expected to change with increased soil water holding capacity and clay content. Since the study site is a floodplain, we expect soil clay content to be spatially variable. Hence, the ECa and elevation maps were used to make sure the full range in soil physical properties (as expected with fluvial deposition) was accounted for in the sampling strategy. Stratified random sampling was used to ensure that the samples chosen were representative of the field with its entire range of characteristics.

For this, the field was divided into four strata (Strata 1: low elevation, low ECa; Strata 2: high elevation, low ECa; Strata 3: low elevation, high ECa; and Strata 4: high elevation, high ECa) and the number of samples (total N: 125) for each stratum was proportional to its areal extent [34]. The sampling locations were chosen randomly within the respective stratum and between the two vegetation types. During sampling, the presence of the desired species was confirmed, with no species intermixing.

Soil sampling was conducted in May 2008, using a tractormounted hydraulic soil probe, with a 6 cm inside diameter soil core. Seventy-eight cores were collected from the *A. donax* vegetation (62% of total, representing the area covered by *A. donax*) and the remaining 47 (38% of total) were from the *C. dactylon* vegetation. Each soil core was separated into five depth increments (0–10, 10–20, 20–30, 30–40, and 40–50 cm) resulting in a total of 514 samples. All the soil depths could not be sampled in all sampling locations because of restrictive layers in the soil profile. The number of samples from each depth for the two crops are shown in Table 1. After

Table 1 – Summary statistics of bulk density (g cm $^{-3}$ ) values under two perennial grasses A. donax L. and C. dactylon (L) Pers in floodplains in Texas, US.								
Soil depth in cm	Mean	Median	Standard deviation	Number of samples	Minimum	Maximum		
C. dactylon (L.) Pers								
0-10	1.00	1.00	0.09	46	0.76	1.19		
10-20	1.12	1.14	0.07	45	0.97	1.26		
20-30	1.12	1.10	0.11	37	0.96	1.37		
30-40	1.14	1.10	0.11	31	0.99	1.46		
40-50	1.10	1.08	0.13	25	0.87	1.51		
A. donax L.								
0-10	0.75	0.77	0.17	79	0.44	1.10		
10-20	1.07	1.09	0.09	79	0.81	1.27		
20-30	1.07	1.06	0.08	70	0.86	1.29		
30-40	1.09	1.09	0.10	56	0.86	1.30		
40-50	1.06	1.07	0.11	46	0.77	1.27		

segregation by depth, the cores were air dried at 60 °C in a convection oven until weight of the samples no longer changed (approximately 24 h) and weighted for bulk density determination.

#### 2.3. Laboratory methods

Prior to analysis, soil samples were ground, passed through a 2 mm sieve, and used for soil C measurements. Total C (TC) was measured by dry combustion method [35] and inorganic C (IC) was measured by the modified pressure calcimeter method [36]. Organic C (OC) was calculated from the difference between TC and IC. A Shimadzu TOC-5050 analyzer was used to measure HC, following extraction procedures from Sparling et al. [37] and Gregorich et al. [38]. From each soil sample, 4 g of soil were weighted and 40 mL of water were added to achieve a 1:10 soil to water ratio [37]. The soil and water mixture was heated at 80 °C for 16 h. The soil suspension was centrifuged for 3 min at 2000 rpm (91  $\times$  g), and filtered through a 0.22 µm GV membrane filter (Durapore). The <0.22  $\mu m$  C fraction, measured on the Shimadzu TOC-5050 analyzer, was defined as HC. Ghani et al. [27] and Gregorich et al. [38] separated cold and hot water extractable C, while Sparling et al. [37] used hot water extraction directly, thus measuring both water soluble C and hot water extractable C. The procedure by Sparling et al. [37] is an easy-to-measure indicator of labile soil C and their procedure showed significant correlation with microbial biomass in the soil. Ahn et al. [39] found that 59% of the variability in potential C mineralization was explained by changes in HC concentration, suggesting that HC is an excellent indicator of changes in soil C. Results are reported for both concentration (c) (HCc, OCc, TCc and ICc, respectively in g kg<sup>-1</sup> of soil) and volumetric (v) units calculated based on the bulk density measurements (HCv, OCv, TCv, ICv, respectively in kg  $m^{-2}$ ).

#### 2.4. Statistical analysis

The Shapiro Wilks test for normality showed that the data was non-normal. Therefore, non-parametric analysis of variance (ANOVA) calculated by the Kruskal–Wallis test was used to determine the effect of crop on TC, HC, OC, and IC (both concentration and volumetric content) at all soil depths (PROC NPAR, SAS Institute).

The ratios between different C pools were also calculated and the effect of crop type and soil depth on the ratios was determined using the Kruskal–Wallis test.

#### 3. Results and discussion

#### 3.1. Effect of crop and depth on soil carbon

Soil C pools were significantly different between crop types and soil depths, but the degree of soil C difference between crop types varied among the four C pools (Tables 2 and 3, Fig. 1). The trends in concentrations for different C pools (Cc,  $g kg^{-1}$ , Table 2) were similar to the trends in content (Cv,  $kg m^{-2}$ , Fig. 1); except for ICv at 0–10 and 10–20 cm depths (Fig. 1).

Table 2 – Summary statistics for concentration (g kg<sup>-1</sup> of soil) of various carbon (C) pools under two perennial grasses A. donax L. and C. dactylon (L) Pers in a floodplain area in Texas, US.

Depth	C. dactylon (L) Pers			1	A. donax L.			
(cm)	Mean	Std	Median	Mean	Std	Median		
		enor			enor			
Total carbon (TCc, $g kg^{-1}$ of soil)								
0-10	43.20	1.65	41.65	59.56	1.53	58.50		
10-20	36.94	1.77	35.70	40.80	1.09	42.50		
20-30	33.91	1.96	32.50	37.62	1.18	39.30		
30-40	30.90	1.60	32.60	37.28	1.42	38.80		
40-50	32.38	2.00	33.80	36.57	1.77	33.85		
Hot water extractable carbon (HCc, g $kg^{-1}$ of soil)								
0-10	0.76	0.05	0.69	1.36	0.06	1.24		
10-20	0.44	0.02	0.41	0.67	0.03	0.62		
20-30	0.36	0.02	0.32	0.48	0.02	0.48		
30-40	0.30	0.02	0.28	0.42	0.02	0.40		
40-50	0.31	0.03	0.30	0.40	0.02	0.37		
Organic carbon (OCc, g kg <sup>-1</sup> of soil)								
0-10	12.41	0.68	12.20	23.44	0.96	21.20		
10-20	7.28	0.53	6.70	10.80	0.52	10.50		
20-30	5.98	0.56	5.50	7.81	0.36	7.45		
30-40	4.79	0.51	4.20	7.11	0.45	6.85		
40-50	5.31	0.60	5.20	6.73	0.57	5.70		
Inorganic carbon (ICc, g kg <sup>-1</sup> of soil)								
0-10	30.79	1.20	29.90	36.12	0.99	36.40		
10-20	29.66	1.33	30.60	30.00	0.74	31.40		
20-30	27.93	1.45	27.20	29.81	0.88	31.80		
30-40	26.11	1.16	26.80	30.17	1.08	32.05		
40-50	27.06	1.45	27.90	29.84	1.32	28.65		

Table 3 — Effect of crop type on various carbon (C) pools derived from non-parametric Kruskal–Wallis one way analysis of variance. Two perennial grasses A. *donax* L. and C. *dactylon* (L) Pers growing in floodplains in Texas, US were compared within a given soil depth.

variables", "	Soli depth in cm								
	0-10	10-20	20-30	30-40	40-50				
Carbon concentration (g kg <sup>-1</sup> )									
TCc	< 0.01	0.03	NS <sup>c</sup>	< 0.01	NS				
HCc	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01				
OCc	< 0.01	< 0.01	< 0.01	< 0.01	NS				
ICc	< 0.01	NS	NS	0.02	NS				
Carbon content (kg m <sup>-2</sup> )									
TCv	NS	NS	NS	< 0.01	NS				
HCv	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01				
OCv	< 0.01	< 0.01	< 0.01	< 0.01	NS				
ICv	0.01	NS	NS	0.04	NS				
Ratios									
HCc/TCc	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01				
OCc/TCc	< 0.01	< 0.01	< 0.01	< 0.01	NS				
HCc/OCc	NS	NS	NS	NS	NS				
Bulk density (g cm <sup>-3</sup> )	< 0.01	< 0.01	0.03	NS	NS				

a TC is total C; HC is hot water extractable C; OC is organic C; and IC is inorganic C.

b The subscript c denotes concentration and v denotes volumetric content of C.

c NS = The effects with p > 0.05 were considered non-significant.



Fig. 1 – Four soil C pools showing differences between two perennial grasses A. donax L. and C. dactylon (L) Pers, growing in a floodplain in Texas, US, across soil depths. a) total C: TCv, b) hot water extractable C: HCv, c) organic C: OCc, and d) inorganic C: ICc (Cv is volumetric content expressed as kg m<sup>-2</sup>of soil). Symbols represent the mean and bars represent the standard error.

Both HCc and OCc were significantly higher under A. *donax* throughout the profile. The largest difference between crops occurred at the top 10 cm depth, with 79% higher HCc and 89% higher OCc under A. *donax*, respectively (Table 2). Hot water extractable Cv and OCv were also significantly higher under A. *donax* (Fig. 1). Similar trends were observed for TCv, but the effect was not statistically significant at all depths (Table 3).

Under A. donax, HCv, OCv, and ICv accounted for 1.5, 24.8 and 75.2% of TCv, respectively (Fig. 1). Soils under A. donax showed 43% higher HCv than C. dactylon at 0–10 cm depth and 46% higher OCv at 30–40 cm depth, while the effect was less than 18% for TCv. Even though the trend of higher soil C under A. donax was consistent for HCv, OCv and TCv, the effect was statistically significant for all soil depths in the case of HCv, for the top four soil depths in the case of OCv and only at 30–40 cm in the case of TCv (Table 3). These findings suggest that HC and OC are more sensitive indicators than TC for discriminating between different crop types. Inorganic carbon was generally stable between crop type and soil depths though it was lower in A. donax at the surface (Table 3). This is likely because IC, which is inherited from the soil parent material, is less sensitive to crop type.

For all C pools, the C content decreased with increasing depth (Fig. 1). The crop difference in TC content was more pronounced at deeper layers (18% higher in A. *donax* at 30–40 cm depth vs. 2% and 4% higher in A. *donax* at the top two depths), while the difference in HC content was more

pronounced in surface layers (43% higher in A. donax at 0–10 cm depth vs. 25% higher in A. donax at 20–30 cm depth and 33% higher in A. donax at 40–50 cm depth). Higher HC contents at the surface were likely due to the addition of easily decomposable fine roots and leaf litter in the upper layers, adding to the labile C pools. Higher TC content in A. donax at deeper soil layers can be attributed to the extensive system of large rhizomes produced by A. donax, which are likely to add significant amounts of biomass to soil [20] as well as decreasing populations of decomposers down the soil profile [40].

#### 3.2. Effects of crop and depth on bulk density

Summary statistics of the bulk density values observed in this study is shown in Table 1. Similar to the HC and OC concentrations, bulk density of the top 30 cm soil was also significantly different between the two crops (Table 3), with lower bulk density under A. donax. The difference in bulk density was most pronounced at the top 10 cm depth where A. donax showed 25% lower bulk density than C. dactylon. Though the C concentrations were very high under A. donax, the lower bulk density dampened the C stock differences between crops. For example, at the top 10 cm depth, the HCc was 38% higher under A. donax as compared to C. dactylon, but HCv was only 1.6% higher. This was because the bulk density was 25% lower under A. donax as compared to C. dactylon. These results

indicate the importance of bulk density measurements in carbon stock studies. They also suggest the potential of A. *donax* for improving the soil structure and aeration as well as water storage characteristics.

# 3.3. Soil carbon storage potential of A. donax in comparison to other crop systems

Our results indicate that A. donax has a significant potential for storing soil organic C. Since A. donax has been present in the study site for the past 40 years, it can be concluded that the positive effects of A. donax on soil C are likely to be long-term. On the other hand, replacement of A. donax with C. dactylon consistently and significantly reduced the soil C stocks. The high C stocks under A. donax were probably due to the input of root biomass. The high root biomass under A. donax has been reported by Monti and Zatta [41] who found that total root biomass was higher under A. donax as compared to Miscanthus, Sorghum and Switchgrass. The authors measured root biomass to 120 cm soil depth and reported that root dry weight under A. donax ranged from 300 kg  $ha^{-1}$  at the top 15 cm and 45 kg  $ha^{-1}$  at 105–120 cm depth. The soil C stocks under A. donax were comparable to the values reported for other perennial grasses. Assuming continuous crop coverage, the TC stocks under A. donax were equivalent to 40.1 Mg ha<sup>-1</sup> TC, while the TC stocks under C. dactylon were equivalent to 37.2 Mg  $ha^{-1}$  in the top 50 cm depth. In comparison, Al-Kaisi et al. [42] reported that after 10 years of perennial grasses under no tillage management, switchgrass had 40.7 Mg  $ha^{-1}$  soil C, smooth bromegrass had 47.1 Mg  $ha^{-1}$ soil C, while corn-soybean-alfafa rotation had only 26.7 Mg ha<sup>-1</sup> soil C in the top 15 cm soil depth. Lee et al. [43] reported that a 26-year-old switchgrass stand contained 18.1 Mg ha<sup>-1</sup> soil C in 0–5 cm depth and 16.6 Mg ha<sup>-1</sup> soil C in the 5-10 cm soil depth. The HCc values under A. donax (1.4 g kg<sup>-1</sup> in the top 20 cm) were in the middle range of the HCc values reported in literature for grasslands. For example, Spohn and Giani [44] reported 1.3 g kg $^{-1}$  HCc in the top 20 cm of a permanent pasture in Germany, while another study in Germany reported 1.2 g  $kg^{-1}$  HCc in the top 20 cm of grasslands [45]. In Florida, Ahn et al. [39] reported 0.7 g kg $^{-1}$  HC in the top 30 cm for improved pastures, while in New Zealand, Ghani et al. [27] reported 3.4 g kg<sup>-1</sup> HC in sheep/beef pastures and 3.0 g kg<sup>-1</sup> HC in dairy pastures. Vasques et al. [46] reported

mean HC content of  $0.34 \text{ kg m}^{-2}$  in the top 30 cm for a variety of land uses in an N. Florida watershed. Although our field site shows commonalities in terms of subtropical climate and calcareous-rich parent material when compared to the HC study in N. Florida it must be noted that the studies differ in terms of soil texture and soil hydrology, which modulate soil C accumulation.

#### 3.4. Combining invasive species control with bioenergy production

Tilman et al. [16] suggested that there are three major classes of biomass used for producing biofuels, (i) monoculture crops such as corn, soybean, switchgrass and sugarcane grown on agronomically suitable land, (ii) waste biomass from agriculture, forestry as well as industrial and urban waste, and (iii) high diversity low input perennial grasses. At our study site, an invasive species is being considered as a potential source of biomass for bioenergy production. Using the invasive species, A. donax, for bioenergy has benefits such as reducing the net cost of control measures, increase in soil C storage and the production of energy or liquid fuel. As discussed in the introduction section, it can also help in ensuring proper disposal of the biomass debris for effective control measures. Unlike crop residues, whose removal may result in reduction in soil C and future crop yields (depending on weather conditions and tillage methods) [47-52], removal of invasive species may benefit the ecosystem. Use of available biomass for bioenergy can also be a profitable intermediate measure in areas where control of the species is not feasible. However, further studies on the economics of the transport and other factors are needed before this practice can be recommended.

Small-scale pyrolysis of this biomass at local level for simultaneous production of bio-oil/syngas and biochar is another possibility. It can minimize the transportation costs and produce biochar in addition to the other benefits. Use of this biochar as a soil amendment can significantly improve soil productivity and long-term soil C sequestration [53]. The A. donax shoot and root biomass has been reported to be suitable material for production of activated C (carbon processed with steam or chemicals to make it extremely porous with very high surface area for adsorption) [54,55]. Although accurate estimates of the areal extent of this species are not available, this plant has been declared invasive in seven US



Fig. 2 – Effects of crop and soil depth (cm) on soil carbon (C) concentration ratios under two perennial grasses A. donax L. and C. dactylon (L) Pers, growing in a floodplain in Texas, US. a) Hot water extractable C/total C (%) and b) Organic C/total C (%). Symbols represent show the mean and bars represent the standard error.



Fig. 3 – Relationships between hot water extractable C with total and organic C concentrations (n: 512) under two perennial grasses A. donax L. and C. dactylon (L) Pers, growing in floodplain in Texas, US. Plots include all depths and both crops.

states [23]. For example in California, A. *donax* was reported to cover 4047 ha in the Santa Ana watershed alone [56], which suggest large potential for the use of this species for bioenergy.

#### 3.4.1. Relationships between carbon pools

The ratios of HC/TC and OC/TC were significantly different between the two crops (Table 3, Fig. 2). Both these ratios were significantly higher under A. *donax* at all five soil depths (except 40–50 cm depth for OC/TC), with the top two depths showing the most pronounced crop differences (Fig. 2). In general, all three ratios decreased with increasing soil depth, but the differences in HC/OC ratios between crop types were not statistically significant (Table 3).

The HC values measured in this study showed significant correlation with both OC and TC (Fig. 3), while HC and OC had the strongest correlation (coefficient of determination  $R^2 = 0.79$ ). The strong correlation between HC and OC is likely because HC is an indicator of the labile component of OC. As a result, HC may indirectly indicate the potential changes in OC and it can offer a way to improve our understanding of soil C dynamics. Hot water extractable C has also been shown to have excellent correlations with indices of microbial activity such as microbial biomass C, microbial N, mineralizable N, and total carbohydrates [27,39].

An exponential relationship was observed between HC and TC (coefficient of determination  $R^2 = 0.63$ ), with larger contribution of HC at higher TC values. Ahn et al. [39] also reported responsiveness of HC/TC ratios to land use, although the ratios reported by the authors were higher than those in this study (4.8–7.1% in the study by Ahn et al. [39] vs. 1.09–2.29% in this study for the top 30 cm). Additional results from Sarkhot et al. [34] also suggested that visible/near-infrared spectroscopy can offer a rapid, low cost and reliable way to estimate HC, indicating that HC can be used for future small or landscape scale soil C monitoring studies.

#### 4. Conclusions

The grass A. *donax* exhibited higher soil C storage as compared to C. *dactylon*. Out of four C pools studied, including TC, OC, IC, and HC, the latter one was found to be the most sensitive to changes in crop type and soil depth. The difference in HC storage was most pronounced in the surface soil layer. Carbon concentrations were more significantly different between crops than the volumetric C content because crop type also affected bulk density. The reduced significance in soil C content compared to concentration illustrates the importance of including bulk density measurements for estimating soil C sequestering potential. Further, these results suggest a significant potential of A. donax for soil C sequestration comparable to other perennial grasses such as switchgrass and smooth bromegrass. Since A. donax is declared as an invasive species in some U.S. states, a careful balance between fuel needs and ecosystem service needs to be considered. This manuscript provides information on the potential C storage of this invasive species compared to a common perennial grass used for livestock and forage production. Our accounting of the soil C stocks provides quantitative information for informed, scientifically-based policy decisions.

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