

Article

## Soil Carbon and Nitrogen Stocks of Different Hawaiian Sugarcane Cultivars

Rebecca Tirado-Corbala<sup>1,2\*</sup>, Ray G. Anderson<sup>1,3</sup>, Dong Wang<sup>1</sup> and James E. Ayars<sup>1</sup>

<sup>1</sup> USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Water Management Research Unit, 9611 S. Riverbend Ave., Parlier, CA 93648-9757, USA; E-Mails: ray.anderson@ars.usda.gov (R.G.A.); dong.wang@ars.usda.gov (D.W.); james.ayars@ars.usda.gov (J.E.A.)

<sup>2</sup> Department of Agro-environmental Sciences, University of Puerto Rico, Mayagüez, Puerto Rico, USA

<sup>3</sup> USDA-Agricultural Research Service, U.S. Salinity Laboratory Contaminant Fate and Transport Unit, 450 W. Big Springs Rd., Riverside, CA 92507-4617, USA

\* Author to whom correspondence should be addressed; E-Mail: rebeccatiradocorbala@gmail.com or rebecca.tirado@upr.edu; Tel.: +1-787-370-9179.

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**Abstracts:** Sugarcane has been widely used as a biofuel crop due to its high biological productivity, ease of conversion to ethanol, and its relatively high potential for greenhouse gas reduction and lower environmental impacts relative to other derived biofuels from traditional agronomic crops. In this investigation, we studied four sugarcane cultivars (H-65-7052, H-78-3567, H-86-3792 and H-87-4319) grown on a Hawaiian commercial sugarcane plantation to determine their ability to store and accumulate soil carbon (C) and nitrogen (N) across a 24-month growth cycle on contrasting soil types. The main study objective establish baseline parameters for biofuel production life cycle analyses; sub-objectives included (1) determining which of four main sugarcane cultivars sequestered the most soil C and (2) assessing how soil C sequestration varies among two common Hawaiian soil series (Pulehu-sandy clay loam and Molokai-clay). Soil samples were collected at 20 cm increments to depths of up to 120 cm using hand augers at the three main growth stages (tillering, grand growth, and maturity) from two experimental plots at to observe total carbon (TC), total nitrogen (TN), dissolved organic carbon (DOC) and nitrates (NO<sup>-3</sup>) using laboratory flash combustion for TC and TN and solution filtering and analysis for DOC and NO<sup>-3</sup>. Aboveground plant biomass was collected and

subsampled to determine lignin and C and N content. This study determined that there was an increase of TC with the advancement of growing stages in the studied four sugarcane cultivars at both soil types (increase in TC of 15–35 kg·m<sup>-2</sup>). Nitrogen accumulation was more variable, and NO<sup>-3</sup> (<5 ppm) were insignificant. The C and N accumulation varies in the whole profile based on the ability of the sugarcane cultivar's roots to explore and grow in the different soil types. For the purpose of storing C in the soil, cultivar H-65-7052 (TC accumulation of ~30 kg·m<sup>-2</sup>) and H-86-3792 (25 kg·m<sup>-2</sup>) rather H-78-3567 (15 kg·m<sup>-2</sup>) and H-87-4319 (20 kg·m<sup>-2</sup>) appeared to produce more accumulated carbon in both soil types.

**Keywords:** Hawaii sugarcane; cultivars; soil carbon; soil nitrogen; carbon sequestration; biofuel

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## 1. Introduction

Sugarcane is widely used as a biofuel crop due to its high biomass, ease of conversion to ethanol, and the higher potential for greenhouse gas reductions and lower environmental impacts relative to other biofuels derived from traditional agronomic crops [1,2]. There is an increasing interest in converting sugarcane to biofuel using advanced cellulosic approaches, particularly in the Pacific Basin [3,4]. The Hawaiian Islands have been identified as a potential location for growing biofuels due to the very high potential productivity of Hawaiian sugarcane and the availability of land following large scale closures of sugarcane plantations [5,6]. Several notable climatic factors are in favor for Hawaiian sugarcane productivity and efficiency, including high solar irradiance (>20 MJ·m<sup>-2</sup>·day<sup>-1</sup>), mild maximum daily temperatures (<30 °C), and low vapor pressure deficit (<1.5 kPa) [7,8]. However, management of Hawaiian sugarcane production is challenging due to high variability in soil fertility, farmland slopes, and other elevation/slope/aspect, and climatic aspects [7,9]. In order to achieve high yields, Hawaiian sugarcane production systems have been improved with the use of numerous practices that are distinctive from other major sugarcane growing regions. One of the most distinctive practices is a ~24 month cropping system with a greater rate of biomass accumulation in the first 15 months of growth and sucrose accumulation thereafter [5]. Other important agricultural practices include: tilling (sub-soiling) the soil to 60 cm depth before the seed canes are planted, using local (Hawaiian), high yielding, disease-resistant cultivars, and using natural predators to control insects and pests. Also, the improved farming practices include the addition of water and fertilizers through drip irrigation systems, incorporation of sand and gypsum to improve the physical properties of the soil before planting, and weed removal in the first six months of sugarcane growth (chemically with glyphosate or hexazinone, or mechanically). The practices of inducing ripening at 12 months by depleting nitrogen in the soil and crop, withholding the irrigation and applying glyphosate for desiccation have been used to enhance sucrose accumulation [9]. Putting those practices together has resulted in high sugar yields of up to 35 t·ha<sup>-1</sup> [10].

Hawaiian sugarcane cultivars have been studied for increased yield [5,11], improved pest resistance [12,13], and salinity tolerance [14] in relation to sugar production. However, parameters

relevant to biofuel production, such as total carbon and nitrogen accumulation, nitrogen fertilizer recovery, and soil organic and inorganic carbon sequestration [15] are less understood, particularly since the rise of drip irrigation in the 1980s [16]. These parameters are critical for biofuel production since they affect fossil fuel inputs via nitrogen fertilizers [17], potential emissions of greenhouse gases such as nitrous oxide that counteract the greenhouse gas benefits of reduced carbon emissions [18], water quality and ecosystem services [19], and energy and economic feasibility [20]. Along with organic carbon sequestration, the long cultivation history (>100 years), continuous monoculture [21], and extensive soil amendments may have created conditions for inorganic carbon sequestration or emissions [22] in Hawaiian sugarcane soils that warrant examination. Furthermore, soil organic matter (SOM) is involved in the maintenance of soil quality, sustainability of natural and agricultural systems and the natural balance of greenhouse gases [23]. For example, pre-harvest straw burning reduces SOM [24,25], thereby affecting the chemical, physical, and biological features of soil. Although SOM represents only a small parcel of the total mass of mineral soils, it is essential for many chemical, physical and biological processes of terrestrial ecosystems [26,27].

Baseline data needed to conduct life cycle analyses [17], required to verify greenhouse gas reductions under current biofuel mandates is lacking [28,29]. In this study, we determined the effect of soil type and cultivar on the carbon and nitrogen accumulation and storage across the 24 month sugarcane growth. We hypothesize that Molokai silty clay soils will have better C sequestration potential due to better protection of organic matter in this tropical environment and that cultivars differ in crop yield, carbon sequestration and the contribution to the carbon emissions.

## 2. Materials and Methods

### 2.1. Study Area

The study was conducted on a commercial sugarcane plantation (CSP) on the island of Maui, Hawaii (20°54' N and 156°26' W). We selected two, ~1 ha experimental plots with contrasting soil types: Pulehu series-cobbly silt loam (fine-loamy, mixed semiactive, isohyperthermic Cumulic Haplustolls) and Molokai series-silty clay loam (Very-fine, kaolinitic, isohyperthermic Typic Eutrotrolox) [30]. These soils were selected because both are common soil series encountered in Maui's agricultural lands. Both plots were planted with four different commercial sugarcane cultivars, H65-7052, H78-3567, H86-3792 and H87-4319. The Pulehu series plot was planted on 19 July 2011 and harvested on 9 June 2013. A total of 375 kg of N ha<sup>-1</sup> in the form of Urea (46% N) was applied in 7 applications in the first 300 days after planting (DAP). The Molokai series plot was planted on 23 June 2011 and harvested on 7 May 2013. A total of 345 kg of N ha<sup>-1</sup>, in the form of Urea, was applied in 10 applications in the first 300 DAP. The CSP uses drip irrigation to supplement rain and tried to maximize limited surface and ground water resources [10]. The drip irrigation system consists of drip laterals spaced at 2.74 m intervals with a row of sugarcane planted on both sides of each drip tape at 46 cm distance away from the tape. The system is pressure compensated to 82.7 kPa (12 pounds per square inch) at risers at the head of the tape lines. The discharge rate is 1.58 L/hour/meter of tape (12.7 US gallons/ hour/ 100 feet of tape). In total 2500 mm of water was drip-applied during the two-year growth cycle.

## 2.2. Baseline Soil Properties of the Experimental Plots

At the beginning of the experiment, 12 samples were collected randomly from each soil depth (0–20, 20–40, 40–80 and 80–120 cm) from the both Pulehu and Molokai soils, oven dried at 65 °C for 48 h, ground and sieved through a 2-mm screen. Soil pH was measured in 1:1 solid/DI water suspension [31]. Electrical conductivity (EC) of the soil samples was determined from a 1:1 soil: DI water suspension. Exchangeable macronutrients such as Calcium ( $\text{Ca}^{2+}$ ), Magnesium ( $\text{Mg}^{2+}$ ), Potassium ( $\text{K}^+$ ) and Sodium ( $\text{Na}^+$ ) were measured using 1-molar ammonium acetate ( $\text{NH}_4\text{OAc}$ ) as the extractant (pH 7) [32] and determined using Inductively Coupled Plasma-Optical Emission Spectrometry analysis (Varian, Palo Alto, CA, USA. (Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture). Soil texture was determined by the hydrometer method [33]. Soil bulk density was determined by using a 5.7 cm diameter bulk density soil sampler (0200 Soil Core Sampler from Soil Moisture Equipment Corp., Ventura, CA, USA).

## 2.3. Soil Variables

In each experimental plot, three samples from each soil depth (0–20, 20–40, 40–80 and 80–120 cm) and sugarcane cultivar were collected at tillering, grand growth, and maturity sugarcane growing stages, oven dried at 65 °C for 48 h, ground and sieved through a 2-mm screen. Total carbon (TC) and total nitrogen (TN) contents were determined by dry combustion with a Flash 2000 N and C Soil Analyzer (Thermo Scientific®, Pittsburgh, PA, USA). Organic carbon (OC) was determined after eliminating all the inorganic carbon (IC) in the samples in the form of carbon dioxide ( $\text{CO}_2$ ), after the samples were acidified with 1:1 HCL: DI water in silver container. The soluble organic compounds were then dried and combusted using the Flash 2000 N and C Soil Analyzer (Thermo Scientific®, Pittsburgh, PA, USA). IC was determined by subtracting OC from the TC. Soil carbon and nitrogen stocks ( $\text{kg}\cdot\text{m}^{-2}$ ) were calculated by multiplying the carbon concentration by the thickness of the soil layer (m) and the soil bulk density ( $\text{kg}\cdot\text{m}^{-3}$ ) for each layer.

Dissolved organic carbon (DOC) was determined after saturating the soil with DI water (1:1 soil:DI water) for 24 h, shaken for one hour on a reciprocal shaker, and filtered through a Whatman no. 42 filter. Carbon recovered in the water extract was determined using a Fusion Total Organic Carbon Analyzer™ (Teledyne Tekmar, Mason, OH). Nitrate ( $\text{NO}_3\text{-N}$ ) content (1:1 soil: DI water) was determined by using Nitrate-Nitrite Astoria Pacific 2 analyzer (Portland, OR, USA).

## 2.4. Plant Measurements

In each experimental plot, plant samples from three locations per sugarcane cultivar were collected to measure aboveground and belowground biomass less than two weeks prior to harvest. Aboveground biomass was determined by using two  $\text{m}^2$  rectangular frames. The long dimension (2 m) of the frame was installed along the row. With the aboveground measurements, we only considered the biomass from the cane stalks growing inside the frame (*i.e.*, green tops, dried leaves and trash on the ground) because sugarcane tends to lodge. Before cutting the cane, the plant height and dewlap of three

representative sugarcane plants were measured. Aboveground dry biomass was determined after oven drying the samples at 65 °C for 5 days. Aboveground dry biomass samples were shipped to our laboratory and ground to 2 mm size particles using a grinder (Thomas Scientific 174931 grinder, Swedesboro, NJ, USA). Lignin content analysis of the different sugarcane cultivars was conducted at an independent laboratory (the Soil, Water, and Forage Testing Laboratory at Texas A and M Agri Life Extension Service College Station, TX, USA).

Belowground (root) biomass was measured at the time when aboveground biomass was sampled. Root biomass for each soil depth interval (0–20, 20–40, 40–80 and 80–120 cm) was determined after collecting soil samples at intervals of 0 m (next to the cane row), 0.75 m and 1.5 m from the sugarcane row using a 7 cm diameter mud auger (Signature Series 350.19, AMS Inc., American Falls, ID, USA). Probe method (mud auger) was selected to minimize disturbance to the soil. All soil samples were stored in plastic containers and were frozen in preparation for root sieving. After thawing, soils were hand sieved by using a 1.4 mm mesh sieve (# 14) to ensure the collection of the majority of the roots. Collected roots from each sample were oven dried overnight at 65 °C to determine root dry weight.

### 2.5. Statistical Analysis

The treatments were arranged in randomized complete design that included four sugarcane cultivars and at two soil types. For each experimental plot (Molokai and Pulehu) and sugarcane cultivar, soil samples were collected at matching sugarcane growing stages (tillering, grand growth and maturity). The soil results from the growing stages for each cultivar were compared for: TC, OC, IC, DOC, TN and NO<sub>3</sub>-N. Plant samples from all the sugarcane cultivars, collected two weeks prior harvest, were compared for aboveground biomass and lignin content. The data were analyzed using the Mixed model of JMP Version 10 (SAS Institute, Cary, NC, USA). Two-way analysis of variance (ANOVA) followed by means separation using Tukey's Honestly Significant Difference (HSD) test at  $Pr < 0.05$  was utilized to examine the significant differences among cultivars and between soil types in soil chemical properties and other variables.

## 3. Results

### 3.1. Soil Chemical Properties

Our results showed that both soils, Pulehu and Molokai, are moderately alkaline (*i.e.*, pH = 7.5–8.2), and there were no acidity problems present in the whole soil profile at the experimental fields (Table 1). Pulehu soils have a higher pH than Molokai soils. At deeper soil depths, we observed an increase in pH (*i.e.*, 8.1 to 8.2) for Pulehu soils and a decrease in pH (*i.e.*, ~8.0 to 7.5) for Molokai soils. However, for electrical conductivity (EC), an opposite pattern of pH is observed in both soils (Table 1). Molokai soils have more than twice the EC (*i.e.*, 0.8 to 1.5 dS·m<sup>-1</sup>) and exchangeable sodium (Na<sup>+</sup>) concentration (*i.e.*, 1.7 to 6.8 cmol<sub>c</sub>·kg<sup>-1</sup>) than Pulehu soils for the whole soil profile, while Pulehu soils have higher exchangeable calcium (Ca<sup>2+</sup>) (*i.e.*, 16 to 22 cmol<sub>c</sub>·kg<sup>-1</sup>), cation exchange capacity (CEC) (*i.e.*, 22 to 25 cmol<sub>c</sub>·kg<sup>-1</sup>), and bulk density than Molokai soils (Tables 1 and 2). This high pH and low EC and Na<sup>+</sup> in Pulehu soils can affect the availability of favorable nutrients such as Ca<sup>2+</sup> and potassium (K<sup>+</sup>), (necessary for sugar cell structure) and soil C.

Pulehu soils have approximately 18 times more exchangeable  $\text{Ca}^{2+}$  than magnesium ( $\text{Mg}^{2+}$ ) and, 1.5–2 more exchangeable  $\text{Ca}^{2+}$  than  $\text{Mg}^{2+}$  is observed in Molokai soils (Table 1). Higher total C and N were observed in samples collected from all soil depths from Pulehu soils compared with Molokai soils (Table 2). However, lower values of BD were obtained in Molokai soils (dominated sandy clay loam texture) compared with Pulehu soils (dominated clay texture) (Table 2).

**Table 1.** Soil Chemical Properties of the Experimental Fields.

Soil	Soil Depth	pH <sup>1</sup>	EC <sup>a,1</sup>	[Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	CEC] <sup>a,2</sup>
	cm	1:1	dS·m <sup>-1</sup>					
					-----cmol <sub>c</sub> ·kg <sup>-1</sup> -----			
Pulehu	0–20	8.10 <sup>b</sup>	0.47	19.4	1.30	0.40	0.60	21.8
	20–40	8.19	0.32	21.1	0.90	0.30	0.60	22.9
	40–80	8.23	0.30	22.4	1.80	0.30	0.80	25.3
	80–120	8.23	0.29	16.0	4.40	0.60	2.30	23.4
Molokai	0–20	7.97	0.81	9.16	4.70	0.65	1.69	16.2
	20–40	7.97	0.94	7.69	3.98	0.39	1.60	13.7
	40–80	7.55	1.13	4.11	2.41	0.13	4.12	10.8
	80–120	7.51	1.50	2.69	2.37	0.18	6.84	12.1

<sup>a</sup> EC = Electrical conductivity and CEC = Cation exchange capacity; <sup>b</sup> Mean values from 12 samples;

<sup>1</sup> 1:1 Soil: DI water suspension; <sup>2</sup> 1M Ammonium acetate extraction.

**Table 2.** Soil Total Carbon and Nitrogen and Physical Properties of the Experimental Fields.

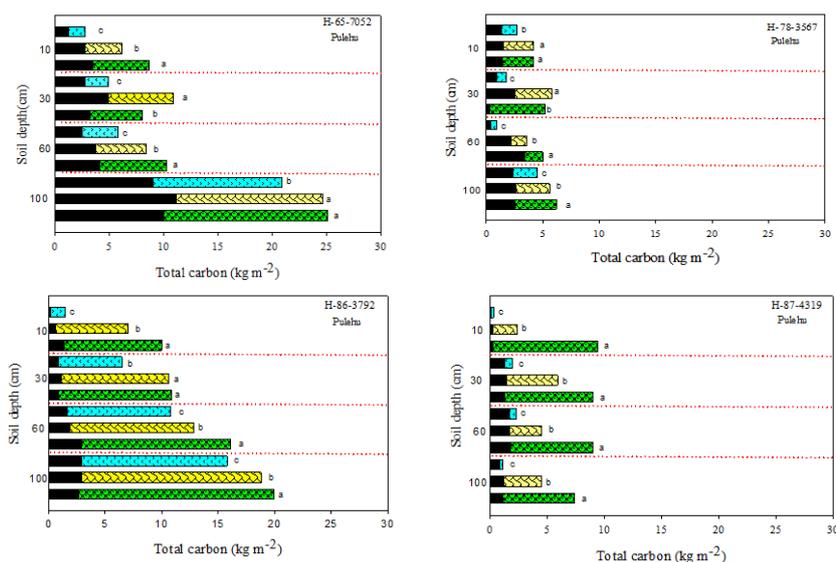
Soil	Soil Depth	TC	TN	BD <sup>a</sup>	Sand <sup>c</sup>	Silt <sup>c</sup>	Clay <sup>c</sup>	Texture <sup>d</sup>
	cm	--- kg·m <sup>-2</sup> ---		---g·cm <sup>-3</sup> ---		-----%-----		
Pulehu	0–20	4.26 <sup>b</sup>	0.90	1.37	53.8	15.8	30.4	SCL
	20–40	3.98	0.78	1.39	58.9	11.6	29.4	SCL
	40–80	5.40	1.04	1.34	43.5	25.2	31.3	C
	80–120	5.25	0.78	1.32	41.6	22.0	36.4	L
Molokai	0–20	2.91	0.31	1.26	27.0	44.2	28.0	C
	20–40	2.86	0.26	1.23	22.0	50.2	27.8	C
	40–80	4.16	0.36	1.26	28.7	46.4	24.8	L
	80–120	3.38	0.21	1.31	36.5	36.6	26.8	L

<sup>a</sup> BD = Bulk density; <sup>b</sup> Mean values from 12 samples; <sup>c</sup> Calculated by using Hydrometer method;

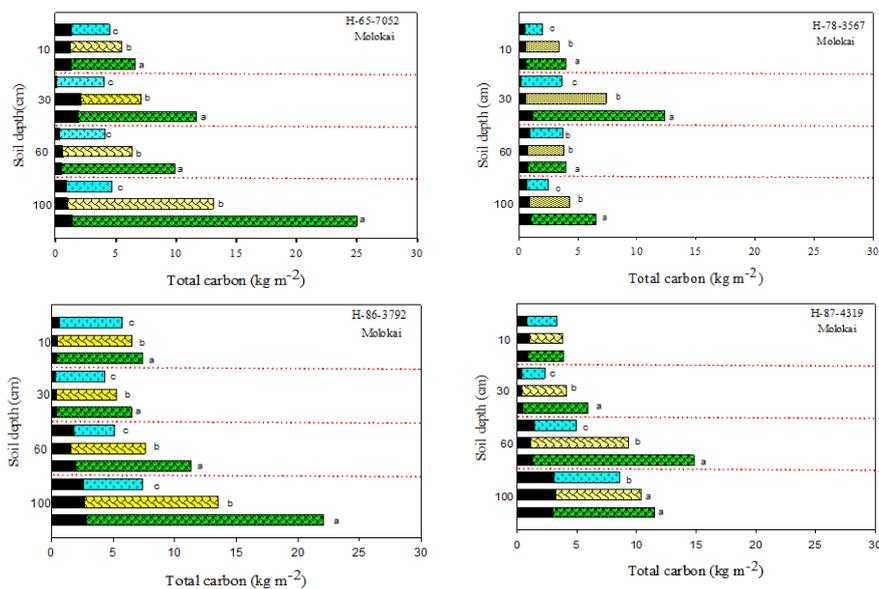
<sup>d</sup> USDA Soil Classification: SCL-sandy clay loam, C-clay, L-loam.

### 3.2. Soil Carbon

In general, total carbon (TC) increased with the growth stages in the four sugarcane cultivars on both soil types (Figures 1 and 2). Cultivar H-65-7052 showed consistent increases in TC ( $P < 0.05$ ) with growing stages for the whole soil profile and at both soil types (Figures 1 and 2). In Pulehu soils, around 45%–60% of the TC of H-65-7052 was in the organic carbon (OC) form and 40%–55% in the inorganic carbon (IC) form. In depth intervals 0–20 and 40–80 cm of Pulehu soils, there was a noticeable increase in OC with the sugarcane growing stages (Figure 1). However, at 20–40 cm depth interval in the Pulehu soils, TC response varied and a higher OC was found during the grand growth stage (Figure 1). At 80–120 cm depth in the Pulehu soils, TC was four times higher than at 0–20 cm depth interval at tillering (Figure 1).



**Figure 1.** Total organic (OC) and inorganic (IC) carbon for the four sugarcane cultivars grown in Pulehu soils. Black (solid) portion of the bars represent the IC and color portion represent OC for each soil depth and sugarcane growing stages (*i.e.*, blue (open arrows)-tillering, yellow (irregular shape)-grand growth, green (light circles)-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different in the Tukey’s test at  $Pr < 0.05$ .

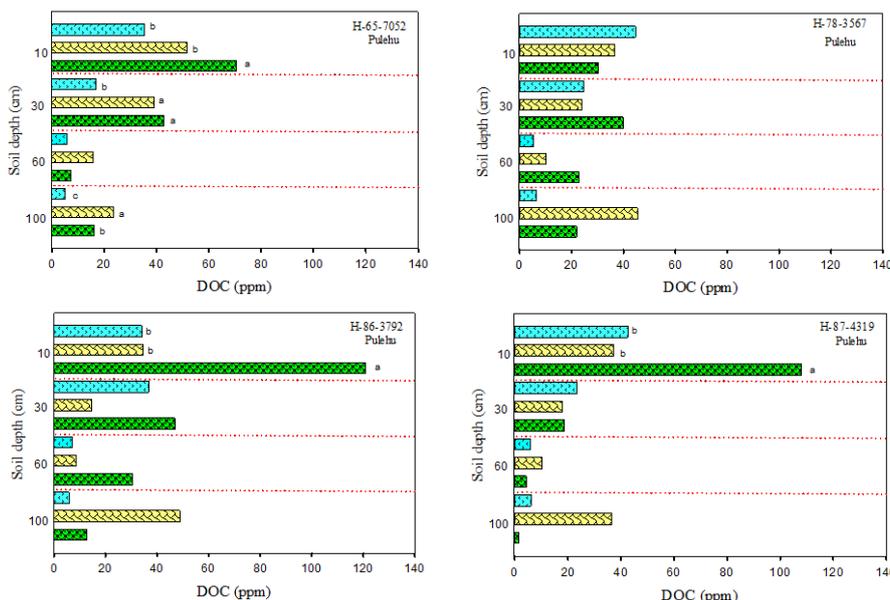


**Figure 2.** Total organic (OC) and inorganic (IC) carbon for the four sugarcane cultivars growing in Molokai soils. Black (solid) portion of the bars represent the IC and color portion represent OC for each soil depth and sugarcane growing stages (*i.e.*, blue (open arrows)-tillering, yellow (irregular shape)-grand growth, green (light circles)-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different Tukey’s test at  $Pr < 0.05$ .

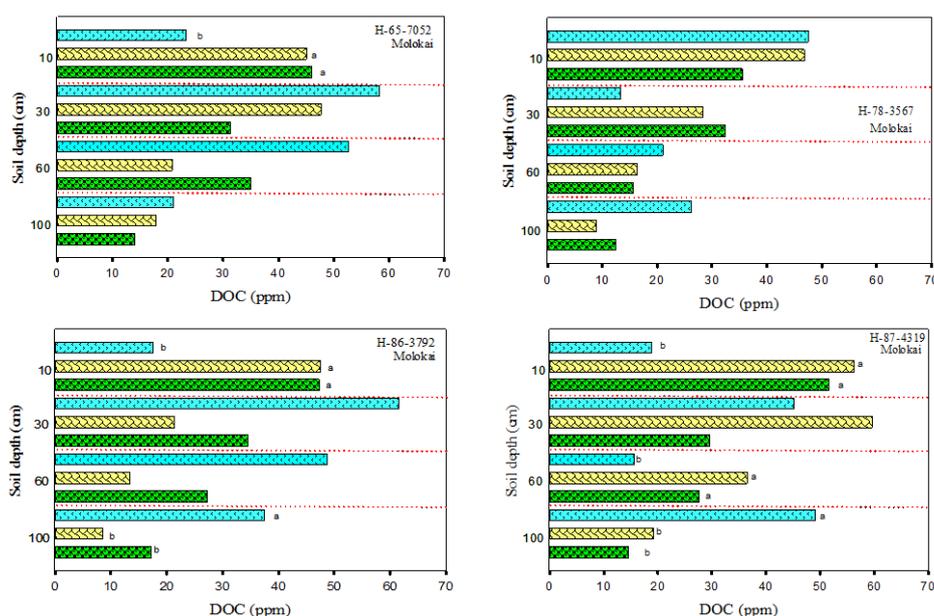
In Molokai soils, cultivar H-65-7052 had more than 70% of the TC in the OC and less than 30% in IC form ( $Pr < 0.05$ ). Also, a 1.5 fold increase was observed in OC from tillering to maturity (Figure 2). In the first 40 cm of soil, TC increases with respect to soil depth and sugarcane growing stages (Figure 2). However, a decrease in TC was observed at 40–80 cm soil depth while the TC increases almost twice at 80–120 cm soil depth from grand growth to maturity (Figure 2).

With respect to dissolved organic carbon (DOC), significantly higher concentrations ( $Pr < 0.05$ ) were found on samples collected from soil depth intervals 20–40 and 80–120 cm at grand growth and maturity stages and 0–20 cm at maturity stage with respect of tillering in Pulehu soils (Figure 3). About twice the DOC concentrations ( $Pr < 0.05$ ) were found on samples collected in the first 20 cm of soil at grand growth and maturity for cultivar H-65-7052 growing in Molokai soils compared with the samples collected on tillering (Figure 4). However at deeper depth, no specific pattern was observed for H-65-7052 growing in Molokai soils (Figure 4).

In the first 20 cm of Pulehu soils, cultivar H78-3567 showed no statistical difference between the samples collected in grand growth and maturity compared with tillering (Figure 1). However, an increase of 1.6 times more OC content was observed over two years growth ( $Pr = 0.04$ ). At depths deeper than 20 cm, there was noticeably higher accumulation of TC (4.4, 6, and 1.4 times more TC at maturity than tillering, for 20–40 cm, 40–80 cm, and 80–120 cm respectively) (Figure 1). Carbon as OC represents more than 50% of the TC in all the samples collected at the different sugarcane growing stages. Significantly higher ( $Pr < 0.05$ ) IC was observed in samples collected at maturity compared with grand growth and tillering growth stages at 20–80 cm depth (Figure 1).



**Figure 3.** Dissolved organic carbon (DOC) in Pulehu soils. Color represents sugarcane growing stages (*i.e.*, blue-tillering, yellow-grand growth, green-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different Tukey's test at  $Pr < 0.05$ .



**Figure 4.** Dissolved organic carbon (DOC) in Molokai soils. Color represents sugarcane growing stages (*i.e.*, blue-tillering, yellow-grand growth, green-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different Tukey's test at  $Pr < 0.05$ .

Cultivar H-78-3567 growing in Molokai soils, showed a 1.2-fold increase in OC collected at grand growth and maturity compared with tillering (Figure 2). The 20–40 cm soil depth interval has the highest observed accumulation of C (threefold increase in OC) from tillering to maturity (Figure 2). However at 40–80 cm soil depth interval, C content remains constant during the whole sugarcane growth ( $Pr = 0.55$ ). At deeper depths ( $> 80$  cm), observed C increases with sugarcane growth stages ( $Pr = 0.02$ ) (Figure 2). Around 71%–95% of the TC in Molokai soils comes from the OC form, while 5%–19% is in the IC form (Figure 2). For DOC, there was no statistical difference between sugarcane growth stages in all soil depths and soil types (Figure 3 and 4). Although, cultivar H86-3792 has a significant increase ( $Pr \leq 0.02$ ) for TC and OC between the growths stages for the whole Pulehu soil profile (Figure 1). More than 80% of the TC in Pulehu soil is OC (Figure 1). However, a higher increase in OC was found in the first 20 cm of soil compared with samples from deeper depths. At deeper depths (21–120 cm), OC increased by 50% or 1.5 times, while inorganic C increased at 40–80 cm soil depth interval ( $Pr = 0.05$ ). Almost twice the amount of IC was encountered at maturity compared with grand growth and tillering (Figure 1), whereas DOC was 3.5 times higher at maturity compared with the samples collected from 0–20 cm depth interval at the other two stages ( $Pr = 0.03$ ) (Figure 3).

Cultivar H86-3792 growing in Molokai soil exhibits the highest amount of TC in the first 20 cm of soil during tillering, and has the least change in TC with depth compared to other cultivars (Figure 2). In the first 40 cm of soil ( $Pr = 0.04$ ), more than 88% of the carbon is present as OC. At deeper depths ( $> 40$  cm), 65%–87% of TC is in OC. At maturity, TC decreases with respect to soil depth in the first 80 cm of soil. However, at deeper depths, there is almost double amount of TC ( $Pr = 0.02$ ) (Figure 2). While for DOC, in the first 20 cm of soil, DOC increases ( $Pr = 0.05$ ) with respect of sugarcane

growing stages. While at 80–120 cm soil depth, lower DOC was found at grand growth ( $Pr = 0.02$ ) (Figure 4).

Cultivar H-87-4319 has consistent increases for TC and OC between the growing stages for the whole Pulehu soil profile (Figure 1) with a wider range of the portion of TC as OC (30%–90%). Cultivar H-87-4319 samples collected on Pulehu soils exhibit lower amounts of C compared with the other cultivars growing in both soils. No statistical difference was found for IC for the whole profile (Figure 1). Whereas, H87-4319 has similar response as H-86-3792 for DOC at the first 20 cm of soil ( $Pr = 0.04$ ). DOC was 2.5 times higher at maturity compared with the samples collected during other two stages (Figure 3).

Cultivar H-87-4319 growing in Molokai soils is the third sugarcane cultivar with higher TC (Figure 2). Irregular response was found in the first 20 cm of soil with respect to sugarcane growing stages for H-87-4319 ( $Pr = 0.25$ ), but consistent increases in TC with growing stage ( $P \leq 0.05$ ) at deeper depths ( $>20$  cm) were observed (Figure 2). An increase in TC was detected with respect to growth stages and more evenly distributed. In the first 80 cm of soil, more than 70% of TC comes from the OC form, while at deeper depths; 64%–74% comes from the OC form (Figure 2). Higher DOC is observed on samples collected from 0–20 and 40–80 cm depth intervals in grand growth and maturity ( $P \leq 0.05$ ) (Figure 4). However at deeper depths ( $>80$  cm), DOC decreases with respect to sampling date. Also, there was a clear reduction of DOC with respect of soil depth in grand growth and maturity compared with tillering stage (Figure 4).

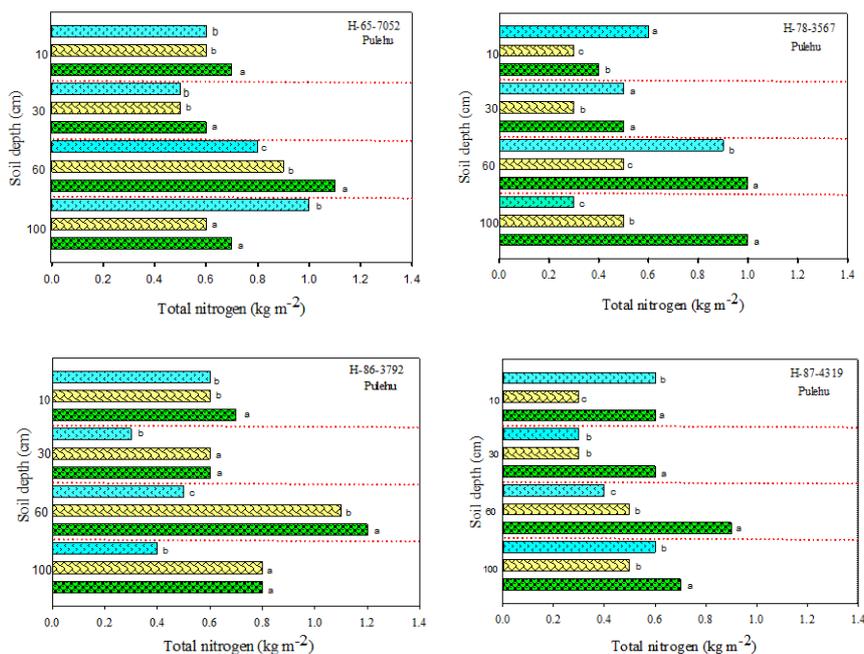
### 3.3. Soil Total Nitrogen and Nitrates

In our study, cultivar H-65-7052 growing in Pulehu soils, higher total nitrogen (TN) ( $Pr \leq 0.04$ ) was observed in samples collected at 20–80 cm depth at maturity (Figure 5). Cultivar H-65-7052 growing in Molokai soils has higher TN ( $Pr = 0.04$ ) in samples collected in the first 40 cm of soil at grand growth (Figure 6). At deeper depths ( $>41$  cm), higher TN ( $Pr \leq 0.04$ ) was measured during tillering (Figure 6). In general, a decrease is observed in TN with respect to depth at grand growth and maturity *versus* tillering (Figure 6).

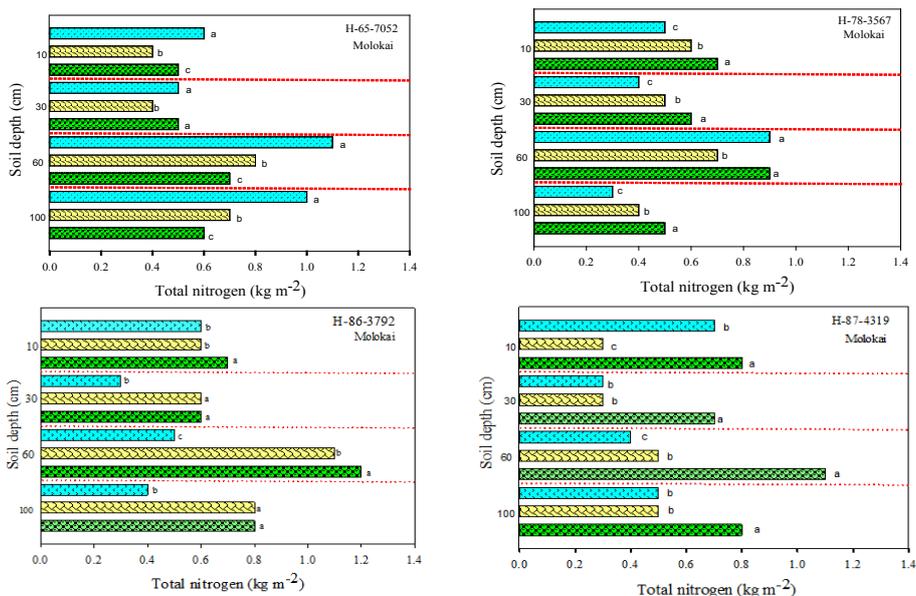
Cultivar H-78-3567 growing in Pulehu soils showed significant variation between growth stages at all soil depths ( $Pr < 0.05$ ); however, there is not a consistent trend between sugarcane growth stages for each soil depth interval (Figure 5). A decrease in TN is observed at tillering with respect of soil depth (Figure 5). In samples collected at grand growth almost the same amount of N is observed in the whole profile. Whereas, on samples collected at maturity, the TN content is observed to increase with respect to soil depth or at deeper depths (Figure 5). However, cultivar H-78-3567 growing in Molokai soils exhibits higher TN ( $Pr < 0.05$ ) on samples collected at maturity in the first 40 cm of soil and 80–120 cm soil depths (Figure 6). Although, no statistical difference ( $Pr = 0.78$ ) was observed for sugarcane H-78-3567 growing in Molokai soils at 40–80 cm soil depth interval. While cultivar H-86-3792 growing in both soils (Figure 5 and 6) and cultivar H87-4319 growing in Pulehu soils exhibits an increase ( $Pr < 0.05$ ) in TN with respect of sugarcane growth stages (Figure 5). Cultivar H87-4319 growing in Molokai soils had increases ( $Pr \leq 0.04$ ) in TN at depths deeper than 20 cm (Figure 6).

Soil nitrate concentration does not exceed 5 ppm in all sampling dates. Maximum  $\text{NO}_3\text{-N}$  concentrations (around 5 ppm), was encountered in the first 20 cm of the Molokai soil samples

collected at grand growth in cultivar H-65-7052 and H-87-4319. This low concentration of nitrate indicates that most nitrates were taken up by the sugarcane or denitrified.



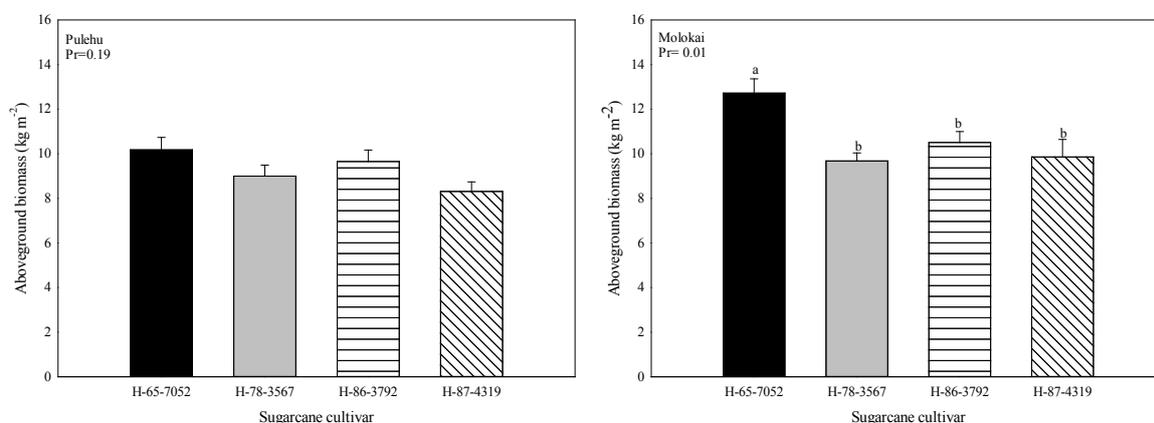
**Figure 5.** Total Nitrogen in Pulehu soils. Color represents sugarcane growing stages (*i.e.*, blue-tillering, yellow-grand growth, green-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different Tukey’s test at Pr < 0.05.



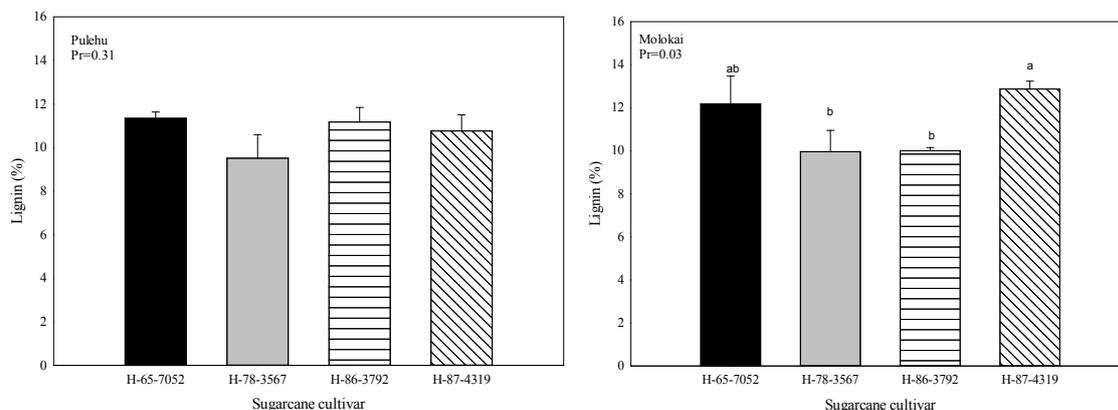
**Figure 6.** Total Nitrogen in Molokai soils. Color represents sugarcane growing stages (*i.e.*, blue-tillering, yellow-grand growth, green-maturity) when the soil samples were collected. Means followed by the same letter or no letters for the bars in each soil depth are not significantly different Tukey’s test at Pr < 0.05.

### 3.4. Aboveground Biomass, Lignin Content, Carbon and Nitrogen in Plant Samples

No water and/or N stress conditions were observed throughout the crop growth cycle (*i.e.*, tillering, grand growth and maturity) in both experimental fields (*i.e.*, Pulehu and Molokai soils). In general, no statistical difference were found for aboveground dry biomass ( $\sim 8\text{--}11 \text{ kg}\cdot\text{m}^{-2}$ ) ( $\text{Pr} = 0.19$ , Figure 7) and lignin content ( $\sim 9\%\text{--}11\%$ ) ( $\text{Pr} = 0.31$ , Figure 8) between sugarcane cultivars growing in Pulehu soils. Even though there was no statistical difference for aboveground biomass between sugarcane cultivars growing in Pulehu soils, it was observed that cultivar H-65-7052 ( $\sim 10.7 \text{ kg}\cdot\text{m}^{-2}$ ) has the highest and cultivar H-87-4319 ( $\sim 8.7 \text{ kg}\cdot\text{m}^{-2}$ ) has the lowest aboveground dry biomass (Figure 7). Similar response to aboveground dry biomass was observed for plant canopy and dewlap of cultivars growing in Pulehu soils. Greater plant canopy height and dewlap length were observed for cultivar H-65-7052 ( $6.8 \pm 0.05 \text{ m}/ 5.8 \pm 0.39 \text{ m}$ ) compared with cultivar H-78-3567 ( $5.6 \pm 0.4 \text{ m}/ 4.3 \pm 0.29 \text{ m}$ ), cultivar H-86-3792 ( $5.1 \pm 0.05 \text{ m}/ 4.0 \pm 0.5 \text{ m}$ ), and cultivar H-87-4319 ( $4.8 \pm 0.2 \text{ m} / 3.36 \pm 0.25 \text{ m}$ ), respectively. Plant moisture content was around 64%–68% on samples collected two weeks prior harvest in Pulehu soils (Data not shown).



**Figure 7.** Aboveground biomass from sugarcane cultivars plant samples collected less than two weeks prior to harvest date. Means from sugarcane cultivars bars followed by the same letter or no letters are not significantly different Tukey's test at  $\text{Pr} < 0.05$ .



**Figure 8.** Lignin content (%) from sugarcane cultivars plant samples collected less than two weeks prior to harvest date. Means from sugarcane cultivars bars followed by the same letter or no letters are not significantly different Tukey's test at  $\text{Pr} < 0.05$ .

In Molokai soils, higher aboveground dry biomass ( $Pr = 0.01$ ) was found for cultivar H-65-7052 ( $\sim 13.8 \text{ kg}\cdot\text{m}^{-2}$ ) compared with the other three sugarcane cultivars ( $\sim 10.5 \text{ kg}\cdot\text{m}^{-2}$ ) (Figure 7). However, higher lignin content ( $Pr = 0.03$ ) was found in cultivar H-87-4319 and H-65-7052 ( $\sim 13\%$ ) compared with cultivar H-78-3567 and H-86-3792 ( $\sim 10.5\%$ ) (Figure 8). A more uniform response was observed for all sugarcane cultivars height and dewlap length: H-65-7052 ( $4.7 \pm 0.05 \text{ m}/ 4.0 \pm 0.20 \text{ m}$ ), H-78-3567 ( $4.9 \pm 0.02 \text{ m}/ 3.5 \pm 0.15 \text{ m}$ ), H-86-3792 ( $4.4 \pm 0.07 \text{ m}/ 3.0 \pm 0.18 \text{ m}$ ) and H-87-4319 ( $4.2 \pm 0.05 \text{ m}/ 3.1 \pm 0.13 \text{ m}$ ). Plant samples collected two weeks prior harvest from the experimental field with the Molokai soil had 35%–38% moisture content.

With respect to plant total carbon (TC) and nitrogen (TN) percentages between cultivars growing in both soils, we observed lower total N (*i.e.*, 0.256- Pulehu and 0.181%-Molokai soils) and variable C (*i.e.*, 50.9- Pulehu and 49.2%- Molokai soils) percentages for cultivar H-65-7052 growing in both soils compared to the other cultivars in each soil type (Table 3). Cultivar H-78-3567 has the 2nd highest amount of total N (0.334%) and C (50.6%) when it grows in Pulehu soils compared with Molokai soils (0.277% and 46.8%, N and C, respectively) (Table 3). Total plant nitrogen and lignin contents are important variables in determining N mineralization kinetics in the soil. When a high C/N ratio is present, intense N immobilization is expected. The N present in sugarcane residues follows a slow decay rate once deposited in the soil which varies from 3% to 30% in one year. Based on our Total C and N results in plants presented in Table 3, we can observe cultivar H-86-3792 has the lower C/N ratio followed by cultivar H-87-4319, H-78-3567, and H-65-7052 on Pulehu soils. Cultivar H-65-7052 has the highest C/N ratio when grows in both soils.

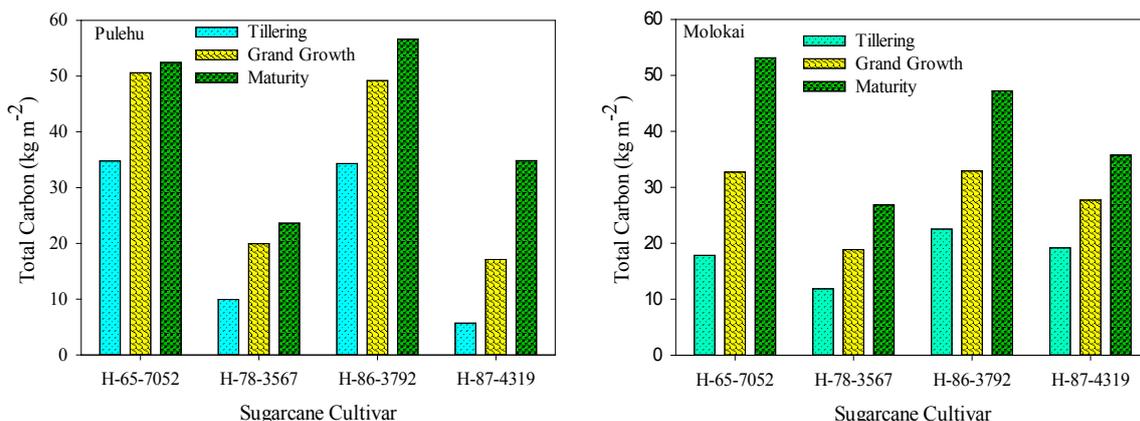
**Table 3.** Total Nitrogen and Carbon and C/N Ratio of Sugarcane Cultivars Plant Samples Collected Less Than Two Weeks Prior to Harvest Date.

Sugarcane Cultivar	Total Nitrogen		Total Carbon		C/N Ratio	
	Pulehu	Molokai	Pulehu	Molokai	Pulehu	Molokai
	-----%-----		-----%-----			
H-65-7052	0.256 c <sup>1</sup> BC	0.181 c D	50.9 a A <sup>2</sup>	49.4 a B	199 a B	273 a A
H-78-3567	0.334 b A	0.277 a B	50.6 b AB	46.8 b C	151 b C	169 c C
H-86-3792	0.367 a A	0.214 b CD	50.4 b AB	50.1 a Ab	137 c C	234 ab A
H-87-4319	0.328 b A	0.232 ab CD	49.2 c B	49.2 a B	150 b C	212 b B

<sup>1</sup> Lower case letter represents difference between sugarcane cultivars within the field (*i.e.*, field with Pulehu or Molokai soils) for each analyzed parameter at  $Pr < 0.005$ ; <sup>2</sup> Upper case letter represents difference sugarcane cultivars between fields for each analyzed parameter at  $Pr < 0.0001$ .

### 3.5. Soil Carbon Accumulation

In general it was observed that cultivar H-65-7052 and H-86-3792 accumulated more TC ( $\sim 50 \text{ kg}\cdot\text{m}^{-2}$ ) than H-78-3567 and H-87-4319 ( $\sim 25$  and  $35 \text{ kg}\cdot\text{m}^{-2}$ , respectively) in the two year sugarcane cycle in both soil types. However, soil total carbon increases (from tillering to maturity) 17% and 36% for cultivar H-65-7052 growing in Pulehu and Molokai soils, respectively (Figure 9). TC increases to around 22% on cultivar H-86-3792 and  $\sim 15\%$  on cultivar H-78-3567 growing in both soils. Therefore, cultivar H-87-4319 has the ability to store more carbon in Pulehu soils ( $\sim 29\%$ ) than Molokai soils ( $\sim 17\%$ ); even though at the end the accumulation is  $\sim 35 \text{ kg}\cdot\text{m}^{-2}$  in both soils.



**Figure 9.** Total Carbon ( $\text{kg}\cdot\text{m}^{-2}$ ) in the first 120 cm of soil accumulation in the different sugarcane cultivars over the sugarcane growing stages.

Soil organic carbon accumulation from tillering to maturity changes with the inclusion of the root carbon fraction (Table 4). Opposite responses ( $\text{Pr} < 0.0001$ ) in soil carbon accumulation were observed for cultivars H-65-7052 and H-87-4319 growing in different soil types (Table 4). The predominant cultivar H-65-7052 has the highest and second lowest accumulation ( $\text{Pr} < 0.0001$ ) of soil organic carbon compared with the other cultivars when grown in Molokai soils ( $\sim 34 \text{ kg}\cdot\text{m}^{-2}$ ) and Pulehu soils ( $\sim 13 \text{ kg}\cdot\text{m}^{-2}$ ), respectively (Table 4). Cultivar H-65-7052 can store 2.6 fold increases in carbon on Molokai soils ( $\sim 34 \text{ kg}\cdot\text{m}^{-2}$ ) compared with Pulehu soils ( $\sim 13 \text{ kg}\cdot\text{m}^{-2}$ ). While cultivar H-87-4319 can store 1.7 fold increases in carbon on Pulehu soils ( $\sim 29 \text{ kg}\cdot\text{m}^{-2}$ ) compared with Molokai soils ( $\sim 17 \text{ kg}\cdot\text{m}^{-2}$ ). Cultivar H-86-3792 stores similar amounts of soil organic carbon ( $\sim 18\text{--}20 \text{ kg}\cdot\text{m}^{-2}$ ) when grown in both soils. While, cultivar H-78-3567 is the lowest organic carbon accumulator ( $\sim 8\text{--}10 \text{ kg}\cdot\text{m}^{-2}$ ) when grown in both soils (Table 4).

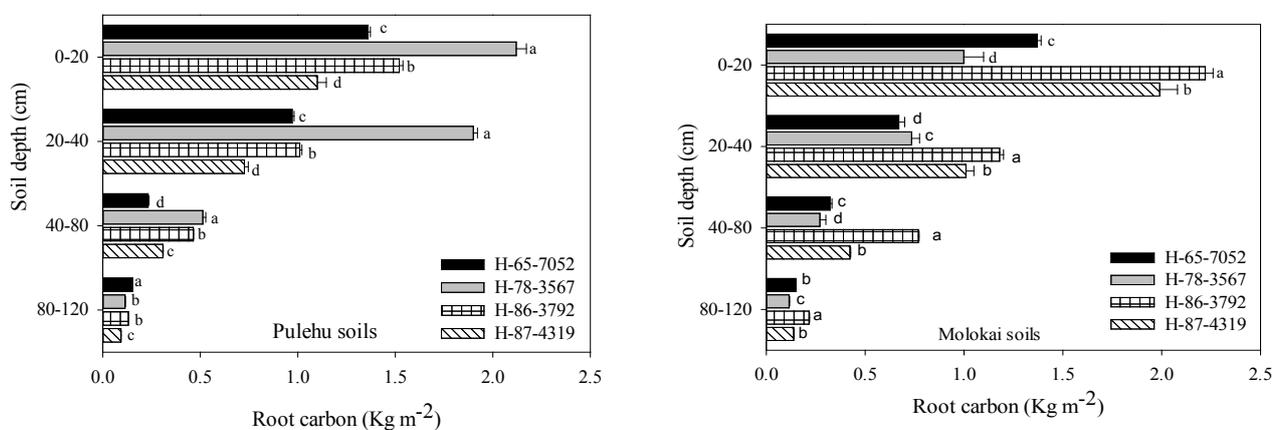
**Table 4.** Soil Organic Carbon Accumulation In Soil from Tillering to Maturity \*.

Sugarcane Cultivar	Pulehu Soils	Molokai Soils
	----- $\text{kg}\cdot\text{m}^{-2}$ -----	
H-65-7052	12.65 c <sup>1</sup> E	33.62 a A <sup>2</sup>
H-78-3567	8.23 d G	10.42 c F
H-86-3792	20.34 b C	18.44 b D
H-87-4319	29.01 a B	17.18 b D

<sup>1</sup> Lower case letter represents difference between sugarcane cultivars within the field (*i.e.*, field with Pulehu or Molokai soils) at  $\text{Pr} < 0.0001$ ; <sup>2</sup> Upper case letter represents difference sugarcane cultivars between fields at  $\text{Pr} < 0.0001$ ; \* Total Carbon in maturity stage includes soil and roots carbon.

Figure 10 shows root carbon content from root samples collected two weeks prior to sugarcane harvest after two years of growth. We found significantly different root carbon density ( $\text{Pr} < 0.05$ ), but no consistent trends, between cultivars for all soil depths and soil types (Figure 10). Our results have demonstrated different root C content are based on cultivar’s root distribution growing in contrasting soil types (*i.e.*, Pulehu vs. Molokai soils). Moreover, our results confirmed that a cultivar’s root distribution is affected by soil texture. Cultivar H-65-7052 exhibits similar response in both soils on samples collected in 0–20 ( $\sim 1.4 \text{ kg}\cdot\text{m}^{-2}$ ) and 80–120 ( $\sim 0.15 \text{ kg}\cdot\text{m}^{-2}$ ) cm soil depth intervals,

respectively. However, for 20–80 cm soil depths, higher root C was observed on Pulehu soils ( $\sim 1.2 \text{ kg}\cdot\text{m}^{-2}$ ) compared with Molokai soils ( $\sim 1.0 \text{ kg}\cdot\text{m}^{-2}$ ). Total root C for cultivar H-65-7052 was  $2.5 \text{ kg}\cdot\text{m}^{-2}$  in Molokai soils and  $\sim 2.7 \text{ kg}\cdot\text{m}^{-2}$  in Pulehu soils. While for the rest of cultivars, there is no consistent or similar response in both soils. Cultivar H-78-3567 exhibits higher root C (sum 0–120 cm) when grown in Pulehu soils ( $\sim 4.7 \text{ kg}\cdot\text{m}^{-2}$ ) compared with Molokai soils ( $\sim 2.1 \text{ kg}\cdot\text{m}^{-2}$ ). Cultivar H86-3792 and H-87-4319 exhibits higher root C (sum 0–120 cm) when grown in Molokai soils ( $\sim 4.4, 3.6 \text{ kg}\cdot\text{m}^{-2}$ ) compared with Pulehu soils ( $\sim 3.1, 2.2 \text{ kg}\cdot\text{m}^{-2}$ ), respectively.



**Figure 10.** Root carbon ( $\text{kg}\cdot\text{m}^{-2}$ ) from sugarcane cultivars collected less than two weeks prior harvest date. Means from sugarcane cultivars bars followed by the same letter or no letters are not significantly different Tukey's test at  $Pr < 0.05$ .

## 4. Discussion

### 4.1. Impacts of Hawaiian Sugarcane Practices Compared to Other Sugarcane Growing Regions

Nutrient efficiencies are crucial agricultural concerns in many regions of the world [34]; especially in tropical settings where the soils tend to be highly weathered. It is well known that the amounts of soil nutrients, such as carbon and nitrogen, are affected by climate factors, management practices (*i.e.*, tilling, liming, fertilization, burning, and irrigation type), vegetation and soil type [35,36]. Rozeff [37] reported that EC greater than  $3 \text{ dS}\cdot\text{m}^{-1}$  may represent a problem tissue sugar concentration [38] and biomass in sugarcane [39,40]. Watcharapirak [41] estimated C storage in various growing stages of Thai sugarcane. The author found that C storage in plant and soil increased with sugarcane growth stages by storing C in the soil, plant, and ground cover. In sugarcane, C was mostly stored in stems ( $1700\text{--}5150 \text{ kg}\cdot\text{ha}^{-1}$ ) at all the growing stages, but the C accumulation rates varied with soil properties and farming management (*i.e.*, fertilization, amendment and irrigation). More carbon is stored in well managed fields [41]. The present study showed a correlation between biomass stored in plants and the quantity of C stored in primary stems [41]. Also, they found that C accumulation rate in the soil increased from tillering to stalk elongation stage (grand growth) and then decreased with plant maturity. In our study, higher organic C content at Molokai series (Oxisol) can be attributed to clay protection of the organic matter as reported by Dominy *et al.* [42]. In Brazil, similar results were found in very clayey [43] and medium-textured soil [44], but not in sand-textured soil [45]. Additionally, a recent review by Cerri *et al.* [46] shows that non-burned areas accumulate more carbon in top soil than

burned areas, and this accumulation depends on soil texture with a 3-fold higher accumulation in clayey than in sandy soil. Robertson and Thorburn [47] showed that soil organic C and total N at 10 and 25 cm soil depth were up to 21% greater than in burned soils. In our study, an increase of C based on sugarcane growing stages was up to two years of growth; however, this pool is affected again by the following burning of cane. According to Robertson and Thorburn [47] and Souza *et al.* [44], crop residues that could be returned to soil are lost in fires, preventing accumulation of nutrients and organic matter from litter and thereby compromising C sequestration and microbial activity in the soils. Also, Thorburn *et al.* [48] found changes in soil C concentrations are highly site specific and not in proportion to the residues were retained (*i.e.*, soil C decrease (up to 2.5 cm)) by  $0.9 \text{ g}\cdot\text{kg}^{-1}$  and  $0.5 \text{ g}\cdot\text{kg}^{-1}$  at sites where residues had been retained for one and 17 years, respectively, but increased by  $2.0 \text{ g}\cdot\text{kg}^{-1}$  at a site with residues retained for six years.

Plant burning produces C in charcoal form, which is non-reactive biologically and chemically when compared to other organic materials of soil, thus representing an inert carbon fraction in C-cycling models [49]. A 57-year study on a Cambissolo (Inceptsol) soil in northern Rio de Janeiro state, Brazil, managed with pre-harvest sugarcane burning crops exhibited TOC levels of  $13.3 \text{ g}\cdot\text{kg}^{-1}$  at a depth of 0–20 cm and  $11.80 \text{ g}\cdot\text{kg}^{-1}$  at 20–40 cm [50]. Compared to these values, the TOC levels detected in the present study were higher in the 10- and 20-year crop areas but lower in the 1- and 5-year crop areas. Sugarcane crops managed with straw burning for 50 years in the northern São Paulo state contained between  $15.4$  and  $19.2 \text{ g}\cdot\text{kg}^{-1}$  of soil TOC at the 0–40 cm depth in clay textured Latossolo Vermelho Distroférrico (Oxisol) and  $6.0$ – $8.4 \text{ g}\cdot\text{kg}^{-1}$  in an area with loamy-sand textured Argissolo Vermelho-Amarelo Distroférrico (Ultisol) [51]. The TOC values found in the present study were higher in the area with loamy-sand textured soils. Pre-harvest sugarcane burning in crops planted in Latossolo Vermelho-Amarelo Distrófico (Oxisol) produces lower TOC than crude sugarcane with or without straw incorporation, with no differences between the two last techniques [44]. In Cambissolo (Inceptsol) areas, long-term sugarcane cropping without straw burning produces 78% higher TOC in the 0–20 cm soil layer and 48% higher carbon in the 20–40 cm layer than in areas managed with pre-harvest straw burning [50]. In sugarcane managed with pre-harvest straw burning, Correia and Alleoni [25] found higher TOC ( $22.7 \text{ g}\cdot\text{kg}^{-1}$  at the 0–5 cm depth and  $20.8 \text{ g}\cdot\text{kg}^{-1}$  at the 5–10 cm depth), probably because the area of the present study exhibited higher sand content. Comparing sugarcane crops managed with straw burning for 55 years with non-burned crop areas, Canellas *et al.* [50] found a 40% decrease in TOC stocks in top soil and 35% decrease in the subsurface layer. Canellas *et al.* [50] argued that sugarcane straw burning promoted SOM oxidation and exposed the soil surface to erosion, thereby decreasing TOC stocks over time. We showed that carbon stocks in the area with 1-year sugarcane crop were not different from those in pasture areas at the depths of 10–20 cm and 20–30 cm. Reductions in carbon stocks were also found in pasture and Cerrado areas. These results indicate that the longer the period of time with stalk burning management, the greater the losses of C stocks. The values of bulk density (Bd) (Table 2) were higher in areas planted with sugarcane than those without sugarcane, leading to increased Bd content and decreased soil aggregation and water infiltration because of soil compacting. Given the increase in soil resistance, root penetration becomes more difficult compromising root system development and ultimately diminishing crop yield in areas with high Bd. In fact, productivity in 2009 and 2010 was 113 and 110 Mg biomass  $\text{ha}^{-1}$  in areas under 1-year old crop; 111 and 106 Mg  $\cdot\text{ha}^{-1}$  for five-year old crop;

85 and 108 Mg·ha<sup>-1</sup> for 10-year old crop and 96 and 85 Mg·ha<sup>-1</sup> for 20-year old crop [47]. The increase in soil Bd, such observed in the present study, is commonly observed in areas converted from natural vegetation into cropland [52]. In such cases, soil aggregates are broken by soil tilling and agricultural machines, causing organic matter loss.

#### 4.2. Relative Nitrogen Uptake Efficiency and Potential for Nitrate Leaching in Hawaiian Sugarcane

Large amounts of N in the form of Urea (345 to 375 kg of N·ha<sup>-1</sup>) have been applied to our experimental fields at the CSP plantation in Hawaii, to minimize crop stress and maximize biomass growth and sugar production. Historically, recommendations of N application rates have been 150–200 kg·ha<sup>-1</sup> in the US [53] and ~ 220 kg·ha<sup>-1</sup> of N globally [54]. Kwong and Deville [55] found the active period of N uptake by sugarcane occurs in the first six months after N application. However, less than 50% of the annually applied N fertilizer is taken up by the sugarcane crops [56,57]. Historically, low efficiencies of N uptake in sugarcane crops were observed in South Africa (9%–31%) [58] and in Taiwan (10%–25%) [57]; however, Australian sugarcane cultivars are able to recover between 6%–54%. Chang and Wang [57] found differences in N uptake efficiency between cultivars. A non-responsive cultivar recovered only 24% of the N *versus* a responsive cultivar which is able to recover 45% of the N. A further sample was observed in Hawaii [58], where one cultivar (H-49-3533) showed a linear response in N uptake over a wide range of fertilizer levels while the other cultivar (H-50-7209) showed a typical non-linear response.

Nitrogen (N) pollution is considered one of the major threats to ecosystem integrity and biochemical cycles on sugarcane plantations [59]. When sugarcane is burnt either pre or post-harvest, 70%–95% of the dry matter and N are lost from the system [60], with nitrate (NO<sub>3</sub>-N) leaching being one of the main pathways [61]. The magnitude of leaching varies with soil type, cropping system, weather conditions, and fertilizer regimes [62,63]. Nitrate leaching is associated with percolation of water and fertilizer application [36]. However, less information in regard to N leaching is available for tropical and sub-tropical, undeveloped agricultural regions. Nitrate produced through nitrification processes in the upper layers can subsequently move downward and accumulate in deeper layers [64,65]. However, in our study, no statistical difference was found for dissolved nitrogen in the form of nitrates (NO<sub>3</sub>-N) in the whole soil profile (0–120 cm) (Data not shown). The NO<sub>3</sub>-N concentration (around 5ppm) found in the present study was similar to those encountered in grassland temperate regions [36] and nitrate concentration increases in the soil profile (up to 120 cm) with the increase of N fertilizer (150–350 kg·ha<sup>-1</sup>) and irrigation. Maximum nitrate level 18 ppm accrued in 350 kg·ha<sup>-1</sup> of N at deeper depth (30–60 cm- clay loam) and irrigation treatment. With passing time (*i.e.*, sampling dates with respect of growing stages) the nitrate concentration increased at deeper depths (clay loam to clay) and decreased in the upper layers (sandy clay loam 0–30 cm).

#### 4.3. Root Dynamics and Soil C Sequestration

Root systems play an important role in the organic matter and nutrient dynamics of the sugarcane growth [66]. In harvest systems without burning, most of the organic matter is returned to the soil with trash harvesting and reincorporation into the soil, whereas in burned systems, the main return of C is through root turnover [67]. Grahan and Haynes [68] found total root biomass was similar under

burning and trashing management practices based on a redistribution of roots towards the first 10 cm of soil in inter-row space versus in row as root proliferated beneath the trash mulch. Also, they found soil C content decreased in response to increasing plant row distance (up to 20 cm) and change of management practice (*i.e.*, burning versus trashing up to 10 cm). Annual C inputs from fine roots frequently equal or exceed those from leaves and can occur to great depths and transfer C deep into subsoil horizons [69,70]. Root systems C exertion ranges from 8% to 26% for sugarcane at 124 DAP, and this response varies depending on sugarcane cultivar, root and air temperature [71]. Rostron [72] established a value of 17% of root system C exertion for South African sugarcane cultivar NCo376 growing under irrigation at 224 DAP. Our study found a similar value of 33% C exertion by the root systems for the four sugarcane cultivars two years after planted. Also, our results demonstrated different root C content are based on cultivar's root distribution growing in different soil texture. Van Anterwerpen [66] found an effect of soil texture on well watered sugarcane cultivar NCo376 root distribution per depth interval. They found highest root biomass in the first 45 cm of clay soil while in sandy soil it was at the 45–75 cm depth. At deeper soil layers (*i.e.*, 75 to 120 cm) there was no difference in root biomass between soil types. Smith *et al.* [73] found that the maximum rooting depth of sugarcane varied with genotype.

## 5. Conclusions

This study evaluated the efficacy of Hawaiian sugarcane cultivars in the accumulation and storage of carbon and nitrogen across the two years of sugarcane growth cycles on two contrasting soils. At both soils, total carbon was increased with the advancement of growing stages in all the four sugarcane cultivars. The carbon (*i.e.*, total, organic and dissolved organic carbon) and total nitrogen accumulation varied in the whole profile (up to 120 cm) depending on the ability of the sugarcane roots to explore and grow in the different soils. Nitrate concentration did not exceed 5 ppm in all sampling dates for the four Hawaiian sugarcane cultivars growing in both soil types; the low concentration of nitrate indicates that most of the applied nitrogen was taken up by the sugarcane plant or little being leached. Based on the results we recommend that the selectively use of sugarcane cultivars with improved traits (such as the cultivars H-65-7052 and H-86-3792 evaluated in the study) can help improve soil carbon and nitrogen cycles, provided that improved farming practices are employed.

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## Author Contributions

Dong Wang and Rebecca Tirado- Corbalá designed the experiment with feedback from James Ayars. Rebecca Tirado-Corbalá conducted most field sampling and analysis in the experimental work with sampling assistance and feedback from Ray Anderson. Rebecca Tirado-Corbalá and Ray Anderson wrote the manuscript, and all the authors revised the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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