1 **Effects of Nitrogen Fertilization and Bioenergy Crop Type on Topsoil Organic**

2 **Carbon and Total Nitrogen Contents in Middle Tennessee USA**

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Abstract

Nitrogen (N) fertilization affects bioenergy crop growth and productivity and consequently carbon (C) and N contents in soil, it however remains unclear whether N fertilization and crop type individually or interactively influence soil organic carbon (SOC) and total N (TN). In a three-year long fertilization experiment in switchgrass (SG: *Panicum virgatum* L.) and gamagrass (GG: *Tripsacum dactyloides* L.) croplands in Middle Tennessee USA, soil samples (0–15cm) were collected in plots with no N input (NN), low N input (LN: 84 kg N ha^{-1} yr^{-1} in urea) and high N input (HN: 168 kg N ha^{-1} yr^{-1} in urea). Besides SOC and TN, the aboveground plant biomass was also quantified. In addition to a summary of published root morphology data based on a separated mesocosm experiment, the root leachable dissolved organic matter (DOM) of both crops was also measured using archived samples. Results showed no significant interaction of N fertilization and crop type on SOC, TN or plant aboveground biomass (ABG). Relative to NN, HN (not LN) significantly increased SOC and TN in both crops. Though SG showed a 15–68% significantly higher ABG than GG, GG showed a 9.3–12% significantly higher SOC and TN than SG. The positive linear relationships of SOC or TN with ABG were identified for SG. However, GG showed structurally more complex and less readily decomposed root DOM, a larger root volume, total root length and surface area than SG. Collectively, these suggested that intensive N fertilization could increase C and N stocks in bioenergy cropland soils but these effects may be more likely mediated by the aboveground biomass in SG and root chemistry and morphology in GG. Future studies are expected to examine the root characteristics in different bioenergy croplands under the field fertilization experiment.

Introduction

Perennial switchgrass (SG: *Panicum virgatum* L) and gamagrass (GG: *Tripsacum dactyloides* L) are two important bioenergy crops that are common alternative energy sources for sustainable replacement of fossil fuels (1-3). Added together with other cellulosic biofuel crops, these dedicated energy crops will contribute to more than 30% of biofuel plant biomass in the coming decades (2, 4). N fertilizers generally increase bioenergy crop yields (5, 6), but many studies report highly varied magnitudes and signals of soil C and N contents in response to N fertilization (7-10). Few studies have compared the root traits in different bioenergy crops and no study has investigated the role of root traits in mediating bioenergy crop and soil responses to N fertilization. Elucidating the effects of fertilization on plant and soil C and N dynamics will provide fundamental knowledge needed to develop effective strategies to improve soil quality, C sequestration, agricultural productivity, and climate change adaptation (11-13).

Past studies showed no consistent pattern of N fertilization effect on SOC and TN contents. N fertilizations can enhance SOC and TN contents by 9–45% in SG croplands (8, 14-20). Under an intensive N fertilization regime (e.g., 180 kg N ha^{-1} yr^{-1}), SOC enhancement is reported due to C accretion from elevated root C input and reduced input of particulate organic C (7, 21). In another study, both inorganic and manure N fertilizations can improve SOC sequestration capacity in SG croplands (15), which is associated with elevated shoot and root biomass (22). In other studies, N fertilizations, however, show no significant effects on SOC pools in a soil profile (0–100cm) at a fertilization rate between 0 and 220 kg N ha^{-1} yr^{-1} (17, 23, 24). A similar conclusion was reached in a fertilization experiment of short-term period of 2–3 years after SG establishment (20). On the other hand, little change in soil TN under

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75 fertilization can be derived based on the N budget for annual SG production, which

76 was closely balanced with 6.3 g N m^{-2} removed by harvest of aboveground biomass

77 and 6.7 g N m^{-2} supplied by fertilization (25). Though not common, N fertilizations

78 can also diminish SOC and TN stocks, and this effect is particularly evident in the

79 stable, mineral-associated C and N pools at depths greater than 15 cm (8). To our best

80 knowledge, no studies have reported soil C and N storages in response to N

81 fertilization in GG croplands. It also remained unknown whether there was significant

82 interaction of N fertilization and bioenergy crop type on SOC and TN stocks.

83   The large variations of SOC and TN in response to N fertilization are

84 typically attributed to the perennial nature of bioenergy crops and their deep-rooted

85 growth form (18). Relative to SG, GG is reported to possess larger roots, higher root

86 biomass and volume (26), total root length and surface area (27). This contrasting root

87 morphology may favor accrual of SOC and TN (28, 29). Besides the root morphology,

88 plant litter and root chemistry of bioenergy crops may also influence SOC and TN

89 changes under N fertilization. Gil and Fick (30) identified a higher plant biomass C:N

90 ratio for GG than other crops, which correlated strongly with lower net N

91 mineralization and losses thus favoring C and N sequestrations (31). The *in situ* root

92 chemistry (C:N) of bioenergy crops is rarely quantified but our recent work found that

93 both plant shoot and root C:N differed largely between SG and GG based on a

94 mesocosm experiment. These studies focused on plant traits, but provided little

95 information of C and N changes in soil simultaneously so that understanding the

96 interaction of soil and bioenergy crop is hindered.

97   Knowing the abundance of humic-like or protein-like compounds will offer

98 information of chemical recalcitrance (32, 33), but this analysis has not been

99 conducted for root of bioenergy crops. A study revealed that the

100   structural-tissue-dominated slow turnover root C pool concentrated at surface soil

101   horizons in a prairie (34) and this indicated a strong linkage of root chemistry with

102   soil C and N storage. However, no study has simultaneously quantified soil response

103   and root traits (e.g., morphology and chemistry) in order to explore how root traits

104   likely moderate soil responses in bioenergy crops.   Using a bioenergy crop field

105   experiment in Middle Tennessee, we investigate the effects of N fertilization on the

106   elemental characteristics of plant and soil C and N in two bioenergy croplands (SG

107   and GG). N fertilization represents the primary management practice in our research

108   plots with no tillage, plowing, or minor mechanical disturbance applied during the

109   experimental period.

110   Given the different nature of SG and GG roots (i.e., chemistry and

111   morphology), we first hypothesize that there is a significant interaction of N

112   fertilization and bioenergy crop type on SOC, TN and plant aboveground biomass

113   such that N fertilization-enhanced SOC, TN and plant aboveground biomass was

114   more pronounced in GG than SG. Alternatively, there is only significant N

115   fertilization effect. In that scenario, we establish the second hypothesize that the N

116   fertilization effect will be significant only under a high fertilization rate because the

117   low fertilization effect can be masked due to large variations in field measurements.

118   Based on a mesocosm experiment examining the two bioenergy crop (SG and GG)

119   seedlings' characteristics, we set up the third hypothesize that the root leachable

120   dissolved organic matter (DOM) is more structurally complex and less easily

121   decomposed for GG than SG because GG root is larger based on the published data

122   synthesis of root morphology in the same mesocosm study. Although we lack

123   fertilization treatment in the mesocosm study, the root morphology and chemistry of

124   the two bioenergy crops are compared and linked to SOC and TN changes in response

5

125 to N fertilization.

126

127 **Materials and Methods**

128 **Site description, soil and plant sample collections**

129 Initially established in 2011, the bioenergy crop field fertilization experiment

130 is located at the Tennessee State University (TSU) Main Campus Agriculture

131 Research and Education Center (AREC) in Nashville, TN, USA (Lat. 36.12° N, Long.

132 36.98° W, elevation 127.6 m above sea level). Prior to the establishment of

133 switchgrass and gamagrass croplands, the land use type was the mowed grassland for

134 several decades. No fertilizers were applied during the prior land use. Climate in the

135 region is a warm humid temperate climate with an average annual temperature of

136 15.1 °C, and total annual precipitation of 1200 mm (35). The crop type and N

137 fertilization treatments were included in a randomized block design (27, 36). The two

138 crop types were *Alamo* SG (*Panicum virgatum* L.) and GG (*Tripsacum dactyloides* L.).

139 The three N levels included no N fertilizer input (NN), low N fertilizer input (LN: 84

140 kg N ha^{-1} yr^{-1} as urea), and high N fertilizer input (HN: 168 kg N ha^{-1} yr^{-1} as urea),

141 and each treatment had four replicated plots with a dimension of 3 m × 6 m. The low

142 N fertilization rate was determined as the optimum N rate to maximize cellulosic

143 ethanol production in established northern latitude grasslands (37). The high N rate

144 doubled the low rate in order to create appreciable gap and detectable effect between

145 the two levels. The fertilizer was manually applied in June or July each year after

146 cutting the grass. The soil series for the plots is Armour silt loam soil (fine-silty,

147 mixed, thermic Ultic *Hapludalfs*) with acidic soil pH (i.e., 5.97) and intermediate

148 organic matter content of 2.4% (36, 38).

149 In the fertilization experiment, soil samples (0–15 cm) were collected from

6

12 plots (2 crops × 3 N inputs × 2 replicated plots) on June 6, 2015. Within each plot, 24 cores were randomly collected using a spatially explicit sampling design (39) and a total of 288 soil cores were obtained in 12 plots. This soil sampling design has been used to quantify the spatial heterogeneity of soil microbial biomass, SOC and TN in the same experiment (36, 40), and in a former study (41). The soil samples were transported to the TSU lab in a cooler filled with ice packs and were then subsequently stored at 4 ℃ until analysis. Visible roots and rocks were removed from the samples, and soil samples were then passed through a 2 mm soil sieve. SOC and TN concentrations were analyzed using a Costech 4010 elemental analyzer (*Costech analytical technologies Inc.*, Valencia, CA, USA). Although 24 samples were collected and analyzed in each replicated plot (i.e., used to map soil C and N spatial heterogeneity in another manuscript), the mean values of SOC, TN, and C:N were obtained in each plot and applied in the ANOVA test in order to avoid the artificial effect of pseudo-replication (42). This generated 12 samples (3 fertilization × 2 crop × 2 replicate).

Harvesting of SG and GG aboveground (ABG) biomass was conducted twice in four replicated plots under each of three fertilization treatments during June to October in 2014 and 2015. This resulted in 24 samples in each year (2 crops × 3 N inputs × 4 replicated plots). At each harvest, plants were cut 7 inches above the ground using a Carter Mfg. Co plot harvester with flail cutters and mounted module capable collecting biomass fresh weights in the field. In each plot, subsamples of fresh biomass per unit area were dried to constant weight at 70 ℃ using an Oven King industrial capacity dryer (Washington Industrial Corp. Seattle, WA, USA) to determine dry biomass. The unit of biomass was expressed as Mg ha^{-1}. To analyze biomass C and N concentrations, subsamples of dry biomass in 2014 and 2015 were

175   selected and one composited sample was obtained by equal weight of sample for each

176   crop under each fertilization treatment (i.e., NN, LN, and HN). This generated 6

177   samples (3 fertilization × 2 crop). Plant samples were analyzed for C and N

178   concentrations using a Costech 4010 elemental analyzer (*Costech analytical*

179   *technologies Inc*., Valencia, CA, USA).

180

181   **The mesocosm experiment, root sample collection and analysis**

182   The root materials of SG and GG were obtained from the historical archived

183   samples collected from a mesocosm experiment (27). Briefly, the experiment was

184   conducted in the greenhouse of the TSU campus farm in 2015 when the SG and GG

185   seedlings were planted in tree pots for three months. Before planting, seeds were

186   germinated in potting mix (FafardH #2 mix). At the 3– to 4–leaf stage, seedlings were

187   transplanted into 15–cm wide x 41–cm high tree pots (Stuewe and Sons,

188   https://www.stuewe.com/products/treepots.php), each containing 6 kg of soil. The pot

189   pH was set at 6.5, which is similar to the acidic soil pH in the field fertilization

190   experiment (i.e., 5.97). Each treatment was replicated eight times. After 3 months,

191   root samples were cut from below the soil surface and rinsed thoroughly with DI

192   water. Root samples were dried in 70 °C to constant weight. The root traits including

193   surface area, length, and biomass productivity were compared between SG and GG

194   and the results have been published formerly (27).

195   For this study, eight replicated root samples for both crops were selected to

196   analyze the abundance and components of dissolved organic matter (DOM) leached

197   from root samples. This generated 16 samples. This analysis was conducted at the

198   Molecular Eco-Geochemistry laboratory of University of Alabama. For DOM

199   leaching, root powders were mixed with carbon-free ultrapure water at a ratio of

8

200 around 1:8 by mass for most samples. If slurry-like mixture appeared at this ratio,

201 extra water was added until a clear liquid layer appeared. The mixtures were

202 constantly agitated for 42 hours on an orbital shaker at 300 rpm, followed by

203 centrifugation at 4,000 rpm for 20 minutes. The upper liquid layer was carefully

204 transferred to a new vial using a pipette and the leachable DOM in these samples was

205 further analyzed for absorbance and fluorescence properties (i.e., Excitation-Emission

206 Matrix coupled with Parallel Factor Analysis), following the analytical methods

207 described in detail in former publications (33, 43).

208   Here we briefly described the procedures on how to conduct the DOM

209 absorbance and fluorescence property analysis. The absorbance of DOM was

210 analyzed using a UV-1800 Shimadzu spectrophotometer, and the spectra from the

211 wavelength of 190 to 670 nm at a 1 nm interval were collected. Three-dimensional

212 fluorescence excitation-emission matrices (EEM) were analyzed using a Horiba

213 Jobin-Yvon Fluoromax-3 spectrofluorometer, with the reading collected at excitation

214 wavelengths from 240 to 500 nm at 5 nm intervals and emission wavelengths from

215 280 to 538 nm at 3 nm intervals. The EEM spectra were corrected for blanks, the

216 inner filter effect, and the manufacturer's correction factors and subsequently

217 normalized relative to the area under the water Raman peak (44). A series of optical

218 indices are calculated to interpret DOM source and compositional characteristics: 1)

219 slope ratio (S_R) of absorbance of 275–295nm over 350–400nm, which is negatively

220 correlated with DOM molecular weight (45, 46); 2) the ratio of E2: E3 (ratio of

221 absorbance at 250 to 365 nm), which decreases as DOM molecular size increases (47);

222 3) fluorescence index (FI), for which lower values are thought to represent larger,

223 structurally more complex compounds usually produced from terrestrial plant decay

224 (48); 4) the ratio of C to T, which indicates the relative amount of humic-like

9

225 (recalcitrant) versus protein-like (labile) compounds in a sample (32); and 5)

226 humification index (HIX), for which greater values correspond to an increasing

227 degree of humification (49, 50).

228   The parallel factor analysis (PARAFAC) was conducted in MATLAB using

229 the DOMFluor toolbox described in detail by (51), and the final model was validated

230 using the split-half analysis (52). Based on fluorescence excitation-emission matrix

231 (32, 53), the PAFRAC model was used to identify three components – C1 and C2

232 representing protein-like DOM and C3 representing humic-like DOM (S1 Table).

233 Given that tyrosine-like DOM is found to be the first component to decrease in leaf

234 leachate during the senescence (54), a high percent tyrosine-like DOM and low

235 tryptophan-like DOM in a sample indicate more labile SOM to microbial degradation.

236

237 **Statistical analysis**

238   Two-way analysis of variance (ANOVA) was used to test the main and

239 interactive effects of N fertilization and crop type on SOC, TN, and C: N, and plant

240 ABG biomass in 2014 and 2015. Tukey HSD *Post hoc* tests were conducted to

241 compare the means when a main or interactive effect is significant. To conduct the

242 ANOVA, the original data was log transformed if it violated equal variance

243 assumption. The regression plots between SOC, TN and plant ABG biomass were

244 also obtained for SG and GG. One-way ANOVA was used to examine the effect of

245 crop type on the indices of DOM leached from root (S_R, E2: E3, FI, and HIX; CT,

246 Tyrosine-like DOM, Tryptophan-like DOM and Humic DOM). These analyses were

247 conducted using R (55). The significance level was set at $P < 0.1$. This threshold

248 *p-value* was selected to accommodate the likely high variability of initial soil C and N

249 contents at the beginning of the experiment.

10

Results

SOC, TN, C: N, and ABG biomass under fertilization in SG and GG

There was no significant interactive effect of fertilization and crop type on SOC, or TN ($P > 0.1$; Table 1). There were significant effects of fertilization and crop type on SOC and TN ($P < 0.1$; Table 1). Post hoc tests indicated that relative to NN, LN insignificantly increased SOC and TN by 2.5% and 2.8%, and HN significantly increased SOC and TN by 15% and 17%, respectively (Table 2). Relative to SG, GG showed a significantly 9.3% higher SOC and 12% higher TN (Table 2). Last, no significant fertilization or crop type effect on C: N was detected, but their interaction effect is significant ($P < 0.1$; Table 1).

Table 1. *p*-values of two-way ANOVA statistical tests on the main and interactive effects of N fertilization and crop type on SOC, TN and C: N as well as aboveground plant biomass (ABG) in 2014 and 2015 under three fertilization treatments in SG and GG croplands at the fertilization experiment in TSU AERC in Nashville, TN, USA.

Variable	N fertilization	Crop	Crop×N fertilization
SOC	**0.072**	**0.082**	0.878
TN	**0.057**	**0.049**	0.429
C: N	0.401	0.163	**0.059**
ABG (2014)	0.434	**0.097**	0.821
ABG (2015)	0.144	**0.025**	0.463

Bold numbers denote P < 0.1.

Table 2. Mean (±SE) SOC and TN concentrations (%), and C: N as well as their respective coefficients of variance (CV, %) under three fertilization treatments in SG and GG croplands at the fertilization experiment in TSU AERC in Nashville, TN, USA.

Crop	N Fertilization	SOC Mean±SE	CV	TN Mean±SE	CV	C: N Mean±SE	CV
		%	%	%	%		%
SG	NN	1.48 ± 0.005^a	0.45	0.13 ± 0.002^a	1.86	11.05 ± 0.09^a	1.13
	LN	1.56 ± 0.09^a	7.91	0.15 ± 0.01^a	11.23	10.57 ± 0.27^a	3.61
	HN	1.72 ± 0.03^b	2.32	0.17 ± 0.0003^b	0.26	10.27 ± 0.18^a	2.45
GG	NN	1.66 ± 0.11^a	9.30	0.16 ± 0.007^a	6.08	10.10 ± 0.23^a	3.15
	LN	1.66 ± 0.14^a	11.52	0.16 ± 0.02^a	14.04	10.62 ± 0.22^a	2.95
	HN	1.89 ± 0.07^b	5.48	0.18 ± 0.004^b	3.51	10.40 ± 0.13^a	1.73

SG: switchgrass; GG: gamagrass; NN: No N input; LN: Low N fertilizer input (84 kg N ha^{-1} yr^{-1});

HN: High N fertilizer input (168 kg N ha^{-1} yr^{-1})

275   There was no significant fertilization effect or interaction of fertilization and

276   crop type on ABG, but there was significant effect of crop type on ABG in both

277   collections in 2014 and 2015 ($P < 0.1$; Table 1). Post hoc tests indicated a

278   significantly higher ABG by 15–68% in SG than GG (Fig 1). The regression plots of

279   SOC, TN and ABG biomass showed stronger linear relationship of SOC or TN with

280   ABG for SG ($R^2 > 0.86$) than those for GG ($R^2 < 0.41$) (Fig 2).

281

282   **Fig 1.** Mean (±SE) plant ABG biomass (Mg ha^{-1}) under three fertilization treatments

283   (e.g, NN, LN and HN) in SG and GG croplands in 2014 and 2015. There was only

284   significant crop type effect on ABG biomass in each collection year (Table 1). Each

285   bar represents a mean value of four replicates (n=4). NN: no N input; LN: low N input

286   (84 kg N ha^{-1} in urea); HN: high N input (168 kg N ha^{-1} in urea); ABG: aboveground.

287

288   **Fig 2.** Regression plots of mean SOC, TN and plant ABG biomass under NN (□

289   square), LN (Δ triangle) and HN (○ circle) in SG and GG croplands. Plant ABG

290   biomass was referred to the collection in 2015 only. NN: no N input; LN: low N input

291   (84 kg N ha^{-1} in urea); HN: high N input (168 kg N ha^{-1} in urea); ABG: aboveground.

292

293   **DOM chemistry of SG and GG roots**

294   Both S_R and FI values were significantly higher for SG than GG samples

295   (Fig. 3), indicating a lower molecular weight and structural complexity of DOM from

296   SG than from GG. The mean values in C:T and HIX were lower and the mean value

297   in E2: E3 was higher for SG than GG, but no statistically significant differences were

298   detected for these indices (Fig. 3). The percent tyrosine-like DOM was significantly

299   higher and the percent tryptophan-like DOM was lower in SG than GG, however,

13

300 percent humic-like DOM little differed between SG and GG (Fig. 3).

301

302 **Fig 3.** Boxplots of DOM source-composition indices (SR, FI and HIX) and percentage

303 of tryptophan-like compound in leachable DOM in root of SG and GG (N=8). For

304 each panel, the different lowercase letters denote significant difference between SG

305 and GG ($P < 0.05$). Boxplots show medians (line), means (dot), 1st and 3rd quartiles

306 (box, interquartile range or *IQR*), upper and lower extremes (whiskers). The whiskers

307 were determined as equal to or less extreme than 1.5 times *IQR* against 1st and 3rd

308 quartiles, respectively. The definitions of indices and compounds were defined in the

309 *Method*s section.

310

311 **Discussion**

312 **N fertilization enhanced SOC and TN concentrations in SG and GG**

313   Based on our results, we rejected the first hypothesis that the fertilization and

314 crop type interactively influenced SOC and TN. However, we found that N

315 fertilization significantly increased SOC and TN in both SG and GG croplands. This

316 was likely due to the minimal management and mechanical disturbance in our plots,

317 which minimized soil decomposition due to less exposure of below surface soil to air,

318 consequently diminished soil C and N losses in favor of soil C and N accumulations

319 in perennial bioenergy feedstock grasslands (56-58). In other bioenergy croplands

320 with more common practices, i.e., tillage, plowing and mechanical movement as

321 implemented in conventional croplands (e.g., wheat and corn), more pronounced soil

322 C and N losses may occur due to greater soil decomposition and likely reverse the net

323 SOC and TN gains to net losses. Furthermore, N fertilization could significantly

324 depress soil respiration, microbial biomass and extracellular oxidases' activities (59,

14

325 60), which led to slow turnover of soil C and N cycles and overall SOC and TN

326 accretions (59).

327   The fertilizer-elevated aboveground biomass yield and the belowground

328 rhizodeposits may also have contributed to the SOC and TN sequestrations by

329 supplying additional amounts of C and N to the soil (7, 56, 61). This interpretation is

330 supported by the fertilization-enhanced aboveground plant biomass in SG (10–15%)

331 and GG (52–61%) as measured in the same year (e.g., 2015) in this study. Though the

332 belowground root biomass was not qualified in the current fertilization experiment

333 due to technical difficulty (e.g., more than 100cm deep soil excavation and much

334 wider horizontal digging), our literature review showed that bioenergy crop root

335 biomass were not responsive to N fertilization (23, 62). Despite the less response of

336 total root biomass, both SG and GG possessed a significant volume and mass of

337 coarse root and recalcitrant root exudate to soil (7, 63-65), which had longer residence

338 times in soil in favor of soil C sequestration (66).

339

340 **N fertilization effects on SOC and TN depend on fertilization rate**

341   Results from this study supported our second hypothesis that relative to no

342 fertilizer input, fertilization resulted in substantial SOC and TN enrichments only at

343 the relatively high N application rates (168 kg N ha^{-1} yr^{-1}) and less likely so at the low

344 fertilization rates (84 kg N ha^{-1} yr^{-1}). This finding contrasts with other studies that

345 have demonstrated negative effects of relatively high fertilization rates on soil C and

346 N storage (7, 67). These negative effects were interpreted as a result of high fertilizer

347 input causing more abundant soil bacteria and low fertilizer use efficiency, which

348 elevated the C and N losses from the soil system (67). Stewart, Follett (7) documented

349 significant soil C and N accretions even when amended with a relatively low fertilizer

15

350   input rate similar to this study. These accretions were likely driven by a greater return

351   of aboveground and belowground plant materials to soil after harvesting. Collectively,

352   a threshold fertilizer input rate may exist in our research plots as to their effects on

353   soil C and N storage, but precautions should be taken when different soil and plant

354   types need to be accounted for. Nevertheless, a wide spectrum of fertilization intensity

355   of up to 300 kg N ha^{-1} has been reported in published studies (68, 69), future studies

356   should examine the N fertilization effects in a wide range of fertilization input rates.

357   From a pragmatic perspective, given the pressing need for minimizing the adverse

358   impacts of agriculture on environment, a recommendation is to adopt a sustainable

359   agricultural practice and the important measure is to lower the use of N and other

360   fertilizers (70). Therefore, it is imperative to elucidate whether a lower-end threshold

361   of fertilizer input exists so that the fertilizer use would continuously benefit both crop

362   productivity and soil fertility with less adverse impact on environment.

363

364   **SOC and TN storage correlate with different plant traits**

365   Our analysis of leachable DOM from root supported the third hypothesis that

366   GG root contained higher molecular weight and more structurally complex compound

367   than SG root. This result indicates that GG root would be less readily decomposed

368   compared to SG root. SG is known to have a lower specific root length (i.e., root

369   length per unit root biomass) (27, 71) and GG to have larger coarse root biomass and

370   volume (26). Given the contrasting root chemistry and morphology between SG and

371   GG, one expected to see a relatively short turnover time for SG root and much longer

372   turnover time for GG root (34). The slow turnover GG root favored long-term SOC

373   and TN sequestrations (34, 72, 73), likely due to more root-derived organic matter in

374   mineral-associated soil fractions (74) and thus offering explanation of the greater

16

375   SOC and TN stocks in GG than SG as observed in this study. The assumption is that

376   the contrasting root characteristics observed in the mesocosm experiment will remain

377   under the field fertilization experiment. This is likely true because of similar soil pH

378   in the mesocosm and field experiments, which play a key role in root growth and

379   development (75).

380   On the other hand, stronger linear relationships of SOC and TN with

381   aboveground plant biomass was identified for SG and less so for GG. Given the

382   significantly greater aboveground plant biomass of SG than GG, these results indicate

383   that the contributions of aboveground plant biomass to belowground soil C and N

384   stocks via litterfall input and turnover were stronger in SG than GG. Considering the

385   aforementioned relationship of GG root with soil C and N storage, our results

386   revealed that the plant traits that contributed to the soil C and N sequestrations varied

387   with bioenergy crop species. It was the aboveground plant biomass of SG and the root

388   of GG that have showed likely associations with their respective soil C and N

389   sequestrations. Despite the long known beneficial role of bioenergy crops on soil C,

390   this study highlighted the need to further elucidate the role of different plant traits

391   (e.g., aboveground vs. belowground) in regulating soil C and N sequestration (76).

392

393   **Conclusions**

394   This study demonstrated that relative to no fertilizer input, intensive N

395   fertilization (e.g., HN) could significantly increase SOC and TN in bioenergy

396   cropland surface soils (0-15cm). Meanwhile, GG showed significantly higher SOC

397   and TN and significantly lower aboveground biomass than SG. There were strong

398   positive linear relationships of SOC and TN with aboveground biomass in SG, and

399   structurally more complex and less readily decomposed root DOM in GG. This

400 suggested that the intensive N fertilization induced C and N accumulations in soil

401 may be more likely mediated by the aboveground biomass in SG and root chemistry

402 and morphology in GG. Future studies should examine the root characteristics in

403 different bioenergy croplands under the field fertilization experiment.

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Supporting information

S1 Table. Characteristics of the three fluorescence components identified by PARAFAC model and their attributed sources. The modeling method was described in *Method* section.

S2 Table. Dataset of SOC, TN and C: N, plant aboveground (ABG) biomass, and root DOM chemistry.