



# Effect of anaerobic soil disinfestation amendment type and C:N ratio on *Cyperus esculentus* tuber sprouting, growth and reproduction

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## Summary

Anaerobic soil disinfestation (ASD) is a cultural technique primarily targeted for control of soilborne plant pathogens, but can also impact weed propagules. A repeated pot study was conducted to evaluate ASD treatment impact on sprouting and growth of introduced *Cyperus esculentus* (yellow nutsedge) tubers using dry molasses-based and wheat bran-based amendment mixtures at four carbon-to-nitrogen (C:N) ratios (from 10:1 to 40:1) and compared with a non-amended control. The mean percentage of sprouted tubers recovered after ASD treatment was lower for wheat bran-based (42%) than dry molasses-based (65%) amendments, and tuber production was 1.6-fold higher in dry molasses-based than wheat bran-based treatments. The highest percentage of sprouted tubers (79%) and the highest mean production of large tubers (threefold higher than wheat bran-based and 1.7-fold higher than molasses-based amendments) were observed for the non-amended control. Tuber

sprouting was significantly lower from all ASD treatments (regardless of amendment C:N ratio) compared with the non-amended control at a 15 cm burial depth. New tuber production was lowest at C:N ratios of 10:1 and 20:1 and more than twofold higher in the non-amended control. Wheat bran-based amendments reduced above-ground *C. esculentus* biomass compared with the non-amended control and ASD treatments with molasses-based amendments, and reduced below-ground biomass compared with molasses-based amendments. Above-ground biomass was highest at amendment C:N ratio of 10:1, and below-ground biomass was highest at amendment C:N ratio of 40:1 and the non-amended control. ASD treatment with wheat bran-based amendments at lower C:N ratios reduced tuber sprouting and reproduction compared with the non-amended control, but not at rates high enough to use as a primary weed management tactic.

**Keywords:** cultural weed control, yellow nutsedge, horticulture, non-chemical weed control.

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## Introduction

*Cyperus esculentus* L. (yellow nutsedge) is a noxious weed that competes with crops for light, soil nutrients

and soil moisture (Morales-Payan *et al.*, 2003). It can also adversely affect crop plants by serving as a host of fungal plant pathogens and nematodes (Schroeder *et al.*, 1999). *Cyperus esculentus* control is challenging if

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tubers are present in production fields (Stoller & Sweet, 1987; Bohren & Wirth, 2015), and a major concern is its ability to produce large numbers of tubers. Tubers are overwintering structures that can remain viable in the soil under extreme climatic conditions, and a single plant can produce hundreds to thousands of tubers  $\text{m}^{-2}$  in a single season (Tumbleson & Kommedahl, 1961). In polyethylene-mulched production systems typical of many horticultural crops, *C. esculentus* rhizomes also perforate opaque mulch due to sharp leaf tips, thus negatively influencing the efficacy of polyethylene mulching (Daugovish & Mochizuki, 2010).

Historically, the broad-spectrum fumigant methyl bromide, in combination with chloropicrin, was effective in minimising *C. esculentus* interference. However, the phase-out of this fumigant has necessitated new strategies for *C. esculentus* control. Alternative fumigants for control of *C. esculentus* are available (e.g. Devkota *et al.*, 2013), as well as soil- and drip-applied herbicides (Dittmar *et al.*, 2012). Fewer options for weed control are available for speciality crops in organic production systems. Physical and mechanical approaches, such as hand weeding and fallow tillage, often require more labour and limit the growing season. Solarisation with or without organic materials is found to be an effective cultural practice to reduce *C. esculentus* interference, but requires temperatures lethal to tuber survival (Johnson *et al.*, 2007). Organic mulches and biological control practices (e.g. *Dactylaria higginsii* and *Puccinia canaliculata*) can also be options, but the effects of these organisms on *C. esculentus* survival and reproduction have been limited (Morales-Payan *et al.*, 2005) and require significant improvement for commercial application (Li *et al.*, 2003).

Anaerobic soil disinfestation (ASD) involves the incorporation of an organic or carbon (C) amendment to supply a labile C source to microbes in order to create anaerobic conditions in polyethylene-mulched soils. Organic acids (e.g. acetic and butyric acids) formed during anaerobic decomposition of labile C have been reported as major control mechanisms of pathogens in ASD (Momma *et al.*, 2006). ASD was initially developed to control soilborne pathogens (Blok *et al.*, 2000), but has also been studied for effectiveness against weed propagules and *C. esculentus* tubers (Muramoto *et al.*, 2008). However, the limited number of ASD studies on weed suppression and variable results across various organic amendments suggests that further targeted research is warranted (Shrestha *et al.*, 2016). In the case of *C. esculentus* tubers, different C-amendments (primarily cover crop residues) with C rates ranging from 0.5 to 2.5 mg C  $\text{g}^{-1}$  soil and C:N ratios ranging from 14:1 to 42:1 have been shown to reduce sprouting percentages compared with non-amended controls

(McCarty, 2012). However, large variability in suppression of tuber sprouting (35–70%) indicates that ASD may need optimisation with characterisation of amendment C:N ratios to better suppress sprouting, growth and proliferation of daughter shoots and tubers. Appropriate C:N ratio of organic amendments is necessary for subsequent horticultural crop performance (non-legumes), not only under aerobic conditions (Rodríguez-Kabana *et al.*, 1987), but also under anaerobic conditions (Butler *et al.*, 2014), due to impacts on N mineralisation and availability. Moreover, C:N ratio is known to be important for shifts in microbial population structure and decomposition of organic matter (Leite *et al.*, 2017) with N-limited growth occurring with C:N ratios above 20–25:1 (Sinsabaugh *et al.*, 2013). However, it is unknown how microorganism response to C:N ratio could impact tuber sprouting following ASD treatment. Given that enhanced microbial activity and organic acid production during ASD could impact *C. esculentus* tuber sprouting, various C:N ratios were examined for their effectiveness in decreasing *C. esculentus* tuber survival and reproduction. Our hypotheses were that (i) *C. esculentus* tuber sprouting would be least in lower C:N ratios due to a lack of N-limitation on microbial decomposition and fermentation of the added labile C and that (ii) *C. esculentus* growth and reproduction of tubers surviving ASD treatment would be highest in lower C:N ratios due to increased nutrient availability. To test these hypotheses, a growth chamber study was conducted with two organic amendment mixtures (dry molasses-based and wheat bran-based) of substrates commonly used in ASD treatment at four C:N ratios to evaluate impacts on tuber sprouting, growth and reproduction of *C. esculentus*.

## Materials and methods

### Experimental setup

Experiments were conducted in an environmentally controlled growth chamber (ECG-TC2-Controller, Chagrin Falls, OH, USA) in spring 2013, in which the temperature was maintained at 25°C (14 h day) and 15°C (10 h night) to simulate soil temperature regimes that represent the relevant production regions during spring in Tennessee (McCarty *et al.*, 2014), as well as many other warm temperate to subtropical horticultural production regions and regions where ASD is under investigation (Shennan *et al.*, 2014). Soil (Dewey silt loam, fine, kaolinitic, thermic, Typic Paleudult) from the surface horizon at the University of Tennessee's Organic Crops Unit, Knoxville, TN (previously planted with maize), was collected and sieved (<10 mm) to remove organic debris and then mixed with

commercial fine grey sand. The soil:sand (1:1) mixture at collection had a total carbon content of 0.94% and soil pH of 6.8. Treatment factors included two types of organic amendment mixtures: dry molasses-based or wheat bran-based amendments supplemented with high N (soyabean meal) or low N (maize starch) amendments to achieve four amendment C:N ratios (Table 1). Molasses and wheat bran were chosen as relatively widely available agricultural by-products that could be used as organic amendments for ASD treatment and also which vary in relative microbial availability (i.e. molasses as a more easily available substrate than wheat bran). A non-amended, untreated control treatment was also included. The total rate of added C in each amendment mixture was established at 4 mg C g<sup>-1</sup> of soil (Table 1). The experimental design was completely randomised with each of four replicates (pots) of each treatment assigned a random number and then arranged in numeric order in the growth chamber. The experiment was repeated (two experiments, in sequence). The relative biological availability of C in amendments, soil and sand was assessed by determining cold-water extractable C and hot-water extractable C with modification of the procedure described by Ghani *et al.* (2003). Briefly, 2 g of C-amendment samples or 4 g of soil or sand samples were extracted in 40 mL of deionised water for 30 min on rotational shaker at 20°C for cold-water extraction. For hot-water extraction, 40 mL of deionised water was added to sediments obtained after cold-water extraction and incubated in a water bath at 80°C for 16 h before centrifugation to obtain extract. Total organic C in water extracts was measured by acidification and sparging method to eliminate inorganic carbon using a total organic carbon analyser (TOC-VCPH model, Shimadzu, Kyoto, Japan).

### Experimental procedure

An equal amount of sand and soil containing C-amendments was mixed by hand and used to fill 2600 cm<sup>3</sup> pots (12 cm diameter, 23 cm height). Six *C. esculentus* tubers of similar size (average 9.5 mm diameter, 0.5 g mass; Azlin Seed Service, Leland, MS, USA) were buried in each pot, three at 5 cm, and three at 15 cm depth. Pots were saturated with tap water to fill soil pore space and allowed to drain from holes in pot bottoms. Oxidation–reduction potential (ORP) electrodes and temperature–moisture sensors (Combination ORP Electrode, Sensorex, Garden Grove, CA, USA and 5TM Soil Moisture Probe, Decagon Devices, Pullman, WA, USA) were inserted at 10–15 cm depth and immediately covered with black polyethylene mulch (0.032 mm), secured with heavy-duty rubber bands and pots incubated in the growth chamber for 3 weeks. The first trial treatment period was 15 March to 6 April 2013, and the second trial treatment period was 8–29 April 2013.

### Cumulative redox potential

Soil temperature, redox potential and soil moisture were continuously monitored and recorded hourly during treatment using ORP electrodes and an automatic data logging system (CR1000-AM 16/32 multiplexers, Campbell Scientific, Logan, UT, USA) over the 3-week ASD treatment. Due to a limited number of temperature probes available and expected limited variability in soil temperature across pots at the time of the study, soil temperature was monitored only in 20 randomly selected pots while ensuring each treatment was monitored in two replicates. Cumulative soil anaerobic activity was calculated as described in McCarty *et al.*

**Table 1** Carbon amendment mixture component properties and rates applied

Mixture components	Component properties			Rate of amendments* (g kg <sup>-1</sup> of soil)			
	C:N ratio	C (%)	N (%)	10:1†	20:1	30:1	40:1
Dry molasses-based mixture							
Dry molasses	29.7	38.7	1.3	6.4	9.4	10.3	7.7
Soyabean meal	4.8	42.6	8.8	3.6	0.9	0.0	0.0
Maize starch	N/A‡	40.3	0.0	0.0	0.0	0.1	2.6
		Total		10.0	10.1	10.4	10.3
Wheat bran-based mixture							
Wheat bran	13.3	41.7	3.1	7.8	6.4	4.2	3.2
Soyabean meal	4.8	42.6	8.8	1.8	0.0	0.0	0.0
Maize starch	N/A	40.3	0.0	0.0	3.3	5.6	6.7
		Total		9.6	9.7	9.8	9.9

\*C rate established at 4 mg C g<sup>-1</sup> of soil for each pot.

†C:N ratio.

‡N/A = not applicable.

(2014). The data logging system provided raw soil redox potential (RP) values averaged on an hourly basis, and critical redox potential (CRP) was calculated as  $(CRP = 595 \text{ mV} - (60 \text{ mV} \times \text{soil pH}))$ . The absolute value of the difference between CRP and RP value was determined for each RP less than the CRP. Cumulative soil anaerobic activity was then calculated by summing absolute values over the 3-week ASD treatment period.

### Soil properties

At the end of ASD treatment, probes were removed, and a soil sample (~80 g wet wt.) was collected from 0 to 8 cm depth from each pot to evaluate bulk soil properties. Subsamples (~10–30 g) were oven-dried (105°C for 48 h) to determine gravimetric moisture content, and the remaining sample was air-dried and sieved (<2 mm). Soil pH was determined on air-dried samples in 0.01 M CaCl<sub>2</sub> (1:2) using a pH electrode (Orion 3-Star Plus pH Benchtop Meter, Thermo Scientific, Waltham, MA, USA) and is reported with the addition of 0.6 (to relate to soil pH values determined in water). For inorganic soil N and total soil N and C, 5 g of air-dried, sieved (<2 mm) soil was extracted with 1 M KCl for 30 min, centrifuged and filtered (Whatman 42) prior to colorimetric analyses for NH<sub>4</sub>-N and NO<sub>3</sub>-N+NO<sub>2</sub>-N using a microplate spectrophotometer (Powerwave XS, Biotek, Winooski, VT, USA) as described by Sims *et al.* (1995). Air-dried, sieved (<2 mm) and pulverised soil samples were analysed for total N and total C by flash combustion (Flash EA 1112 NC Soil Analyzer, Thermo Scientific). For extractable soil phosphorus, 5 g of air-dried, sieved (<2 mm) soil was extracted with Mehlich-1 extractant for 5 min (Mehlich, 1953), centrifuged and filtered (Whatman-42), and extractant was determined using a malachite green microplate method (D'Angelo *et al.*, 2001).

### *Cyperus esculentus* assessment

After the ASD treatment, polyethylene mulch was removed and pots were incubated in the growth chamber and hand-irrigated with tap water supplied regularly throughout the growing period. Each pot was fertilised with 200 mg N (as blood meal) after ASD termination. After an 8-week period following soil treatment, *C. esculentus* tuber mortality was assessed. Any non-germinating *C. esculentus* tubers were recovered from the soil and assayed visually. The visual inspection of recovered tubers was completed by dissecting the tubers and then examining the internal colour and condition of tubers (Stoller & Wax, 1973; Banks, 1983). Ratings were assigned based on a 1–5

scale, with a rating of 1 indicating tubers that were firm and undecomposed and white, ratings of 2–4 indicating tubers that were soft and in various advancing stages of decomposition, with colours ranging from yellow to red to brown, and a rating of 5 indicating tubers that were completely rotten/decomposed with grey to black colour. *Cyperus esculentus* biomass was assessed by removing above-ground and below-ground biomass from pots and washing of all adhering soil. As it was not possible to accurately differentiate root biomass by tuber burial depth, biomass data are representative of the entire pot. Both below-ground (recoverable roots and tubers) and above-ground biomass of plants were recorded after oven drying at 65°C for 48 h. Newly formed tubers were categorised as small (<0.5 cm) or large (>0.5 cm), based on average dry diameter, and counted.

### Statistical analysis

Data were analysed according to a completely randomised experimental design with a 2 × 4 factorial (carbon source × C:N ratio), with an additional non-amended control treatment. Response variables of environmental conditions (soil temperature, soil moisture, soil pH, anaerobic conditions, soil nitrogen, soil phosphorus) and *C. esculentus* survival and growth (tuber sprouting, *C. esculentus* growth and reproduction) were analysed with the Glimmix procedure and Fisher's P-LSD at  $P = 0.05$  using SAS, version 9.3 (SAS Institute, Cary, NC, USA). C-amendment (dry molasses-based mixture or wheat bran-based mixture) and C:N ratio (10, 20, 30 and 40:1) were treated as fixed effects for factorial analysis, and trial was treated as a random effect (i.e. a random intercept). If the C-amendment by C:N ratio interaction was not significant, data for main effects only are presented. Additionally, main effect treatments were analysed separately to compare each treatment with the non-amended control treatment. Data were checked for normality with a Shapiro–Wilk test and for homogeneity of variances with a Levene test. Only soil inorganic N data did not comply with normality and homogeneity assumptions and thus were subjected to rank transformation. Actual (non-transformed) means and standard error of means are reported.

## Results

### Soil temperature and moisture

Soil temperature at 15 cm depth averaged 25°C and generally ranged from a low of 15°C to a high of 30°C during the treatment period and was consistent



in both trials. Soil moisture content was similar among C-amended pots and non-amended pots in both trials. Average gravimetric soil moisture at the beginning of trials was  $0.08 \text{ g g}^{-1}$ , and at the end of ASD treatment, soil moisture content increased to  $0.23 \text{ g g}^{-1}$ .

#### Cumulative anaerobic conditions and soil pH

Cumulative anaerobic conditions were significantly affected by C:N ratio and C-amendment type, but not the interaction (Table 2). All amended pots showed significantly higher cumulative anaerobic condition than the non-amended control (range from 89322 mV h for C:N ratio of 40:1 to 105548 mV h for C:N ratio of 20:1, Fig. 1A). Mean cumulative anaerobic conditions did not differ between dry molasses-based and wheat bran-based mixtures (Table 2). Soil pH was significantly affected by the main effect of C:N ratio, but not C-amendment or the interaction (Table 2). Soil pH was lowest at amendment C:N ratio of 10:1 (pH 6.1), slightly higher for the non-amended control (pH 6.2) and highest at C:N ratios of 20:1, 30:1 and 40:1 (pH 6.3; Fig. 1B).

#### Soil nutrients

Total soil inorganic N (i.e.  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$ ) was significantly affected by treatment C-amendment and C:N ratio, but not the interaction (Table 3). Following ASD treatment and prior to N fertiliser application, the lowest mean total soil inorganic N was observed from C:N ratio of 40:1 ( $<4 \text{ mg N kg}^{-1}$  soil; Table 3) and highest from amendment C:N ratio of 10:1 ( $60 \text{ mg N kg}^{-1}$  soil). Total soil inorganic N was primarily comprised of  $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$  (76–91% of total inorganic N) rather than  $\text{NH}_4\text{-N}$  (9–24%) at sampling. The non-amended control had the least amount of soil  $\text{NH}_4\text{-N}$ . Soil C:N ratio prior to treatment was 11.7, and after termination of ASD treatment, the highest soil C:N ratio was observed for the non-amended control (12.2 soil C:N ratio) and least for C:N ratio of 10:1 (10.3 soil C:N ratio).

The interaction between C-amendment and C:N ratio was significant for Mehlich-1 extractable soil phosphorus. In treatments with wheat bran-based amendments, extractable soil phosphorus was significantly higher at C:N ratios of 10:1 and 20:1 (38 and  $32 \text{ mg P kg}^{-1}$  soil respectively) than C:N ratios of 30:1 and 40:1 (23 and  $19 \text{ mg P kg}^{-1}$  soil respectively; data not shown). Soil phosphorus did not differ among C:N ratios for molasses-based amendments ( $14\text{--}18 \text{ mg P kg}^{-1}$  soil) and was similar to the non-amended control ( $14 \text{ mg P kg}^{-1}$  soil).

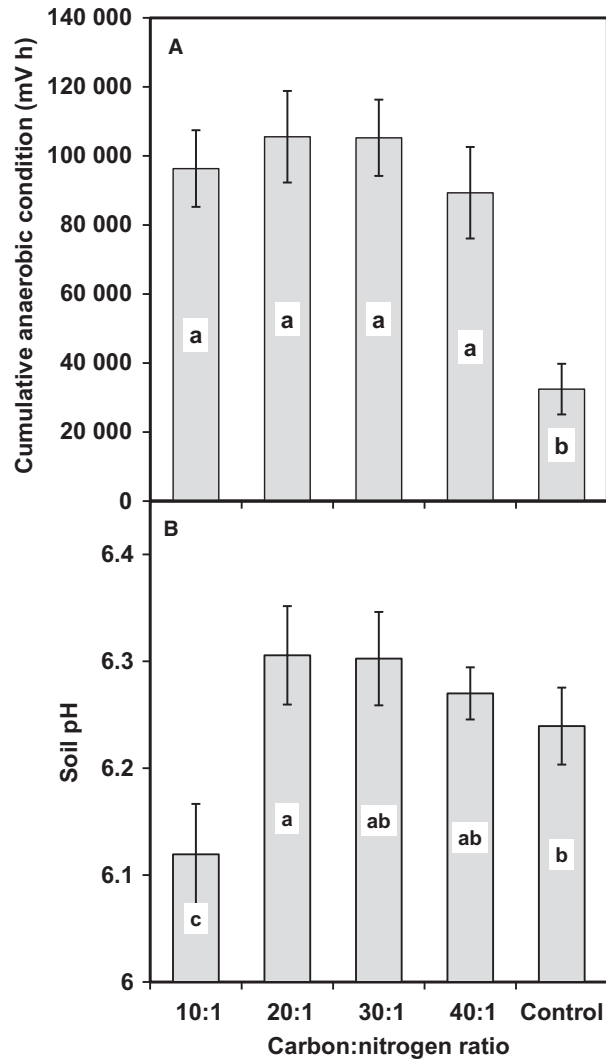
**Table 2** Analysis of variance for response variables of soil constituents and tuber constituents as affected by carbon amendment, C:N ratio and the interaction

Carbon amendment	Soil properties		Tuber sprouting (%)				Tuber production (tubers $\text{pot}^{-1}$ )			Cyperus esculentus biomass (g $\text{pot}^{-1}$ )		
	Soil pH	Cumulative anaerobic condition (V h)	15-cm depth	5-cm depth	Total tubers	Large tubers	Small tubers	Total tubers	Above-ground	Below-ground	Significance (P-value)†	Significance (P-value)†
Dry molasses-based	$5.7 \pm 0.03$	$115 \pm 10.5a^*$	$65.6 \pm 6.3a$	$64.6 \pm 6.4$	$65.1 \pm 4.8a$	$16.5 \pm 2.0b$	$5.1 \pm 0.6$	$21.7 \pm 2.3b$	$11.1 \pm 1.2a$	$9.3 \pm 0.9a$	NS	$<0.001$
Wheat bran-based	$5.6 \pm 0.03$	$105 \pm 7.3a$	$29.2 \pm 5.7b$	$54.2 \pm 6.7$	$41.7 \pm 5.3b$	$9.2 \pm 1.8c$	$3.9 \pm 0.7$	$13.1 \pm 2.2c$	$6.6 \pm 0.9b$	$5.0 \pm 0.8b$	$<0.001$	$<0.001$
Control	$5.6 \pm 0.03$	$50 \pm 12.2b$	$81.3 \pm 6.8a$	$77.1 \pm 7.3$	$79.2 \pm 4.4a$	$28.0 \pm 2.2a$	$5.4 \pm 0.7$	$33.4 \pm 2.4a$	$8.6 \pm 0.5b$	$11.9 \pm 1.2a$	NS	$<0.001$
Carbon amendment	NS	$<0.001$	$<0.001$	NS	$<0.001$	$<0.001$	NS	$<0.001$	$<0.001$	$<0.001$	NS	$<0.001$
C:N ratio	$<0.001$	0.001	0.002	NS	0.01	$<0.001$	0.01	$<0.001$	0.02	0.001	NS	0.001
C-amendment $\times$ C:N ratio‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*Within columns values (mean  $\pm$  standard error) followed by different letters are significantly different according to Fisher's protected LSD test at  $P < 0.05$ .

†Probabilities associated with individual F tests. Data were analysed using mixed model analysis of variance. NS: not significant.

‡Interaction effect does not include control treatments.



**Fig. 1** Effect of anaerobic soil disinfestation (ASD) amendment carbon-to-nitrogen (C:N) ratio on (A) mean cumulative anaerobic conditions and (B) soil pH during ASD treatment. Bars indicated by different letters are significantly different according to an F-protected LSD,  $P < 0.05$ . Error bars indicate standard error.

### Tuber sprouting

During the 3-week treatment period, for most tubers that sprouted, shoots emerged and remained under plastic. However, some shoot emergence through plastic was observed in all covered treatments (31–36% emergence) during the treatment period. Following plastic mulch removal and at the shallower depth of 5 cm at the end of the study, tuber sprouting did not differ significantly among C:N ratios and amendments, ranging from 56% at C:N ratio of 10:1 to 77% for the non-amended control (Fig. 2A). Sprouting of tubers at the 15 cm depth differed significantly among C:N ratios and amendments, but the interaction was not significant (Table 2). The lowest sprouting at 15 cm depth was

observed for wheat bran-based amendments (29%; averaged across C:N ratios) and for C:N ratio of 10:1 (33%; averaged across amendment types), and the highest sprouting for the non-amended control (81%, Fig. 2A). The percentage of total sprouted tubers recovered from wheat bran-based amendments averaged across depths and C:N ratios (42%) was lower than dry molasses-based amendments (65%) and the non-amended control (79%). The mortality of tubers in the wheat bran-based treatments is attributed to a higher number of slightly decomposed to rotted tubers (ratings 3, 4, or 5; 2.9 tubers  $\text{pot}^{-1}$ ) than the number of slightly decomposed to rotted tubers retrieved from molasses-based amendment (1.8 tubers  $\text{pot}^{-1}$ ) or non-amended control treatments (1.3 tubers  $\text{pot}^{-1}$ ; data not shown).

### *Cyperus esculentus* growth and reproduction

*Cyperus esculentus* growth was significantly affected by C-amendment and C:N ratio without any interaction (Table 2). Dry below-ground biomass was greatly reduced by wheat bran-based amendments compared with the non-amended control and similar for above-ground biomass. Between C-amendments, greater above-ground biomass and below-ground biomass were observed for molasses-based amendments. Among C:N ratios, above-ground biomass was highest at C:N ratio of 10:1 and similar among all other C:N ratios and the non-amended control (Fig. 3A). Below-ground biomass was lower for C:N ratios of 20:1, 10:1 and 30:1 compared with the non-amended control (Fig. 3B). The higher below-ground biomass in the non-amended control and C:N ratio of 40:1 is consistent with higher production of new tubers in these treatments (Fig. 2B). No interaction was observed between amendment type and C:N ratio for mean tuber production (Table 2). The number of tubers produced was higher from the non-amended control treatment (33 tubers  $\text{pot}^{-1}$ ), lowest for wheat bran-based amendments (13 tubers  $\text{pot}^{-1}$ ) and intermediate for molasses-based amendments (22 tubers  $\text{pot}^{-1}$ ). Among total tubers, 79% of tubers were large (>0.5 cm) in size and 21% were small (<0.5 cm) in size. The number of small tubers was highest for C:N ratio of 40:1 (7 tubers  $\text{pot}^{-1}$ ), and large tubers were highest in the non-amended control (28 tubers  $\text{pot}^{-1}$ , Fig. 2B). Among C:N ratios, the number of both large and small-sized tubers was lowest for C:N ratio 10:1 (12 tubers  $\text{pot}^{-1}$ ) and greatest for C:N ratio 40:1 (23 tubers  $\text{pot}^{-1}$ , Fig. 2B).

### Discussion

Soil nutrient levels observed after ASD treatments were generally consistent with other reported ASD

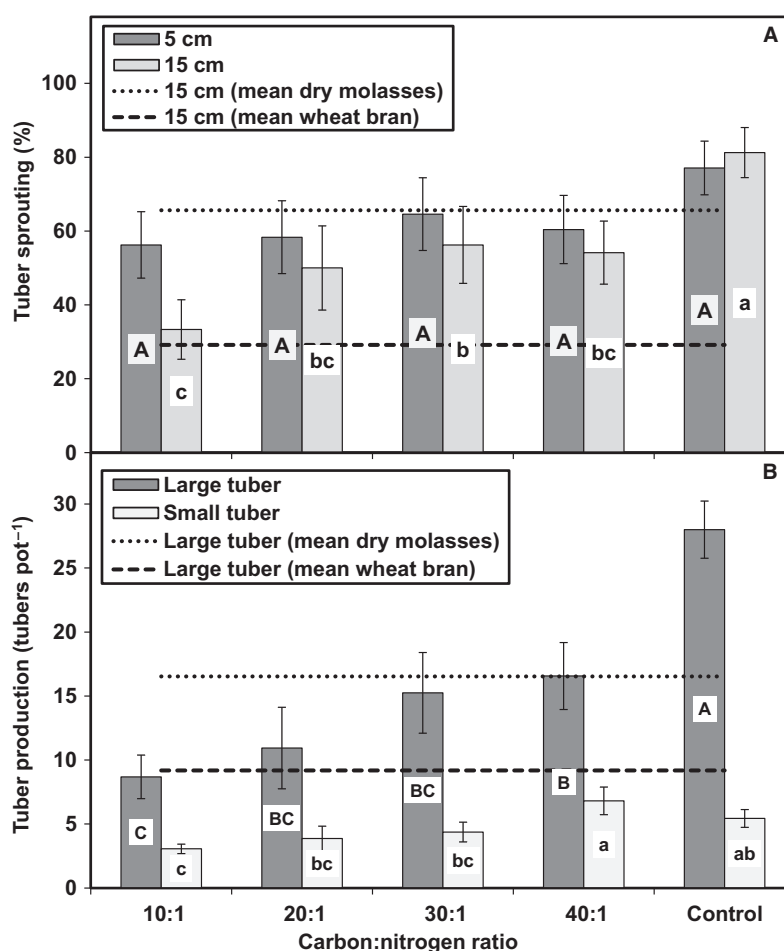
**Table 3** Soil nitrite-N + nitrate-N (NO<sub>2</sub>-N + NO<sub>3</sub>-N), ammonium-nitrogen (NH<sub>4</sub>-N) and total inorganic N as affected by soil amendments and amendment C:N ratios

Amendment	Soil nitrogen (mg N kg <sup>-1</sup> soil)			Soil C:N (ratio)
	NO <sub>2</sub> +NO <sub>3</sub> -N	NH <sub>4</sub> -N	Total inorganic N	
Control	4.7 ± 0.9b*	1.4 ± 0.2b	6.0 ± 0.8b	11.7 ± 0.1a
Dry molasses-based	19.6 ± 4.0a	2.0 ± 0.1a	21.5 ± 4.1a	11.1 ± 0.2b
Wheat bran-based	22.7 ± 4.2a	2.2 ± 0.1a	25.0 ± 4.2a	10.7 ± 0.2b
C:N ratio				
10:1	57.6 ± 2.8a	2.3 ± 0.2a	59.8 ± 2.9a	10.3 ± 0.2c
20:1	19.3 ± 1.3b	1.9 ± 0.2a	21.2 ± 1.4b	11.0 ± 0.1b
30:1	6.4 ± 1.1c	2.1 ± 0.1a	8.5 ± 1.2c	11.2 ± 0.1ab
40:1	1.4 ± 0.6d	2.0 ± 0.3a	3.4 ± 0.6d	11.5 ± 0.3a
	Significance ( <i>P</i> -value)†			
Carbon amendment	0.03	<0.001	0.001	<0.001
C:N ratio	<0.001	<0.001	0.01	<0.001
C-amendment × C:N ratio‡	NS	NS	NS	NS

\*Within columns values (mean ± standard error) followed by different letters are significantly different according to Fisher's protected LSD test at *P* < 0.05.

†Probabilities associated with individual *F* tests. Data were analysed using mixed model analysis of variance. NS: not significant.

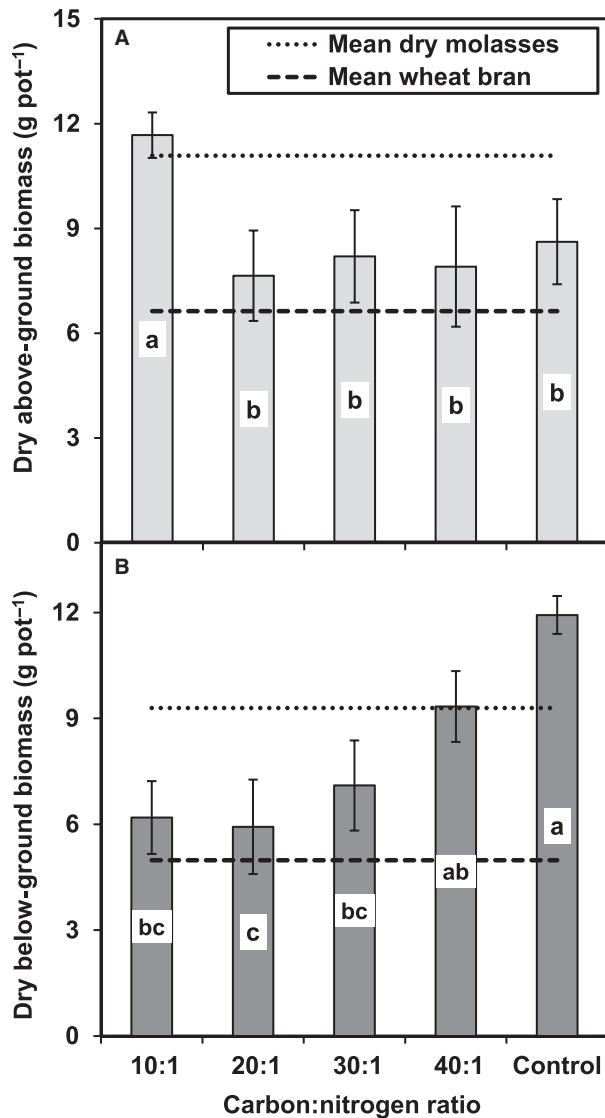
‡Interaction effect does not include control treatments.



**Fig. 2** Effect of anaerobic soil disinfestation (ASD) amendment carbon-to-nitrogen (C:N) ratio on (A) mean *Cyperus esculentus* tuber sprouting at 5 m and 15 cm depths and (B) production of large or small tubers during an 8-week period following ASD treatment, averaged across amendment type. Dashed lines represent mean sprouting or tuber production by amendment type, averaged across C:N ratios. Within depth and tuber size, bars indicated by different letters are significantly different according to an F-protected LSD, *P* < 0.05. Error bars indicate standard error.

research and expected impacts of amendment C:N ratio (Sinsabaugh *et al.*, 2013). Total soil inorganic N was highest for amendment C:N ratio of 10:1

(60 mg N kg<sup>-1</sup> soil) and lowest for amendment C:N ratio of 40:1 (3.4 mg N kg<sup>-1</sup> soil) as more mineralisation of organic N in amendments probably occurred at



**Fig. 3** Effect of anaerobic soil disinfestation (ASD) amendment carbon-to-nitrogen (C:N) ratio on (A) mean dry above-ground and (B) below-ground *Cyperus esculentus* biomass production, averaged across amendment type. Dashed lines represent mean biomass by amendment type, averaged across C:N ratios. Bars indicated by different letters are significantly different according to an F-protected LSD,  $P < 0.05$ . Error bars indicate standard error.

the lower C:N ratio. Total soil inorganic N at treatment termination was comparable with that reported by McCarty *et al.* (2014) in Tennessee. The higher values of extractable soil P for wheat bran-based amendments at lower C:N ratios are primarily due to high P content (81–112 mg P kg<sup>-1</sup>) of these amendment mixtures as compared to higher C:N ratios (41–53 mg P kg<sup>-1</sup>) or dry molasses-based amendment mixtures P (8–32 mg P kg<sup>-1</sup> of amendments).

As higher soil N content at C:N ratio of 10:1 promotes vegetative growth by enhancing basal bulb

formation rather than tuber formation (Garg *et al.*, 1967; Stoller & Sweet, 1987), it was not surprising that both dry below-ground biomass and tuber production were lower at this ratio. Total tuber production was synchronously higher with the below-ground biomass at higher C:N ratios (Figs 2B and 3). Despite having amended with fertiliser N after ASD treatment, only dry molasses-based amendments enhanced the above-ground growth, suggesting that above-ground growth was more influenced by nutrient content of applied amendments and soil N (see Table 3) at these rates of fertiliser application. As N availability becomes limited, allocation of nutrients to tuber production (Chellemi *et al.*, 2013) may account for the larger number of tubers produced in the higher C:N ratios (20:1 and above).

*Cyperus esculentus* tuber sprouting was significantly lower from all ASD treatments (regardless of amendment C:N ratio) compared with the non-amended control at a 15 cm burial depth, but not significantly different among treatments at the shallower 5 cm depth. This suggests that dynamics of ASD treatment at shallower depths have less effect on *C. esculentus* tuber decomposition and mortality, potentially due to a reduced anaerobic period due to diffusion of oxygen and soil drying from the soil surface. While highly anaerobic conditions were generated in our ASD-treated pots, similar to other ASD studies (e.g. Butler *et al.*, 2012; McCarty *et al.*, 2014), no relationship has been established between *C. esculentus* tuber sprouting or emergence and cumulative anaerobic conditions in the few studies that have evaluated *C. esculentus* response to ASD treatments (Muramoto *et al.*, 2008; Butler *et al.*, 2012; McCarty *et al.*, 2014). In the present study, there was similarly only a weak correlation between *C. esculentus* sprouting and cumulative anaerobic conditions (Pearson  $r = -0.21$ ,  $P = 0.06$ ). All ASD treatments had lower soil pH at treatment termination than initial average soil pH (6.8), which may be due to the production of acetic acid and butyric acid (Momma *et al.*, 2006), and the organic acid content may have been higher for C:N ratio of 10:1 as indicated by the lowest soil pH, which could partially explain the dynamics of *C. esculentus* sprouting and reproduction with ASD amendment C:N ratio. After ASD treatment, the presence of high total soil inorganic N from amendment incorporation at the lower C:N ratios (10:1 or 20:1) has the added benefit of increasing relative availability for crop uptake, thus reducing fertiliser requirement and preventing reduced crop performance due to N immobilisation that may occur at higher amendment C:N ratios.

It is likely that the observed tuber sprouting percentage for molasses-based amendments (65% at the 15 cm



depth) is not comparable with the results of Muramoto *et al.* (2008) and Butler *et al.* (2012) because soil temperatures in those studies were higher (>25°C) than in the present study. Overall, the use of wheat bran-based mixtures at lower C:N ratios provided better tuber control than molasses-based mixtures. These amendments not only differ in their nutrient content, but also differ in decomposition rate; dry molasses has more sucrose content and results in faster decomposition, while wheat bran is more fibrous and decomposes more slowly. This difference was observed in cold-water extractable C-amendments (47 mg C kg<sup>-1</sup> versus 12 mg C kg<sup>-1</sup> for dry molasses and wheat bran respectively) and for additional C extracted by hot water (49 mg C kg<sup>-1</sup> versus 6 mg C kg<sup>-1</sup> for dry molasses and wheat bran respectively). Microbial decomposition of these amendments in ASD plays an important role in the production of organic acids. Given that the concentration of acids produced during ASD may not be high enough to cause tuber mortality, as Ozores-Hampton *et al.* (1999) reported that the concentration of acetic acid at <2.5 g kg<sup>-1</sup> from mature compost was ineffective at killing sprouted *C. esculentus* tubers, it is likely that multiple mechanisms are at work, including biological control by microorganisms promoted by ASD treatment.

Tubers buried in plastic-covered, non-amended control treatments produced 92% more tubers per unit area than amended treatments, which was more than fivefold the number of tubers introduced into each pot. While tuber production was much lower in wheat bran-based amendments compared with molasses-based amendments and the non-amended control, and less in low C:N ratios than high C:N ratios, *C. esculentus* tuber production in these treatments was still at a minimum twofold higher than the number of buried tubers. Although temperature may be a limiting factor with regard to tuber mortality, ASD treatment at moderate soil temperatures did significantly reduce tuber density compared with the non-amended control, which could help limit crop yield loss over time if combined with other management strategies. For example, an integrated weed management strategy could potentially combine soil treatment by ASD with mulch barriers less susceptible to piercing by the leaf tips of *C. esculentus* (e.g. Daugovish & Mochizuki, 2010) or soil solarisation during ASD treatment (clear rather than opaque plastic mulch) to increase weed propagule mortality (Johnson *et al.*, 2007; Butler *et al.*, 2014). Conventional herbicide options labelled for *C. esculentus* control in horticultural crops are few and often have efficacy limitations (McAvoy & Freeman, 2013); however, if paired with ASD treatment, this integrated system may provide adequate control (Guo *et al.*,

2017). Similarly, while biological herbicide options may not provide adequate control of *C. esculentus* alone (Li *et al.*, 2003; Morales-Payan *et al.*, 2005), additive and synergistic effects may increase feasibility in ASD-treated systems. Lastly, the reduced sprouting in ASD treatment at the 15 cm depth compared with the 5 cm depth in the present study suggests that for full field (rather than bedded) applications of ASD treatment, repeated shallow tillage or other mechanical operations (Johnson *et al.*, 2007; Hershenhorn *et al.*, 2015) during a post-treatment fallow period could potentially be used to decrease energy reserves of *C. esculentus* propagules at the soil surface.

The results of the present study indicate that ASD treatments can reduce *C. esculentus* tuber sprouting and reproduction, especially at lower ASD amendment C:N ratios, but that ASD as applied in this study will not be an adequate control strategy without other integrated weed management tactics.

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