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RESEARCH ARTICLE

# Feedstock composition influences vermicomposting performance of *Dichogaster annae* relative to *Eudrilus eugeniae* and *Perionyx excavatus*

Micah Martin[1] · Gaius Eudoxie[1]

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## Abstract

Carbon to nitrogen (C/N) ratio influences substrate combinations and earthworm performance in vermicomposting systems. To elucidate these factor effects, a comparative evaluation of species, C/N ratio combined with feed rate, was conducted on three local earthworm species: *Perionyx excavatus*, *Eudrilus eugeniae*, and *Dichogaster annae*. Earthworms were stocked at similar densities and fed shredded paper (SP), cattle manure (CM), and lawn clippings (LC) combined to form C/N ratios of 28, 36, and 53. Earthworms were fed at rates of 1, 1.25, and 2 g feed (dry wt.)/g worm/day for a period of 8 weeks. Percent vermiconversion, earthworm adult and juvenile biomass, and vermicast quality were measured. Vermicast production was significantly affected by the combination of C/N ratio and feed rate and varied among species. All treatment combinations resulted in > 70% conversion, except *E. eugenaie* fed at the medium rate. Vermiconversion increased for *P. excavatus* and *D. annae* with increasing C/N ratio but decreased with increasing the feed rate. Vermicast EC, pH, and C/N ratio was strongly affected by species, relative to other experimental factors. *D. annae* showed the greatest change in biomass, which peaked at the highest feed rate and lowest C/N ratio. Average adult biomass decreased for *P. excavatus* with increasing feed rate, while differences were nonsignificant for *E. eugenaie* and *D. annae*. Significant increases in average juvenile biomass were only evident for *D. annae* in response to increasing feed rates. Feed rate had a greater influence on earthworm population dynamics and vermicast quality compared to initial feedstock C/N ratio.

**Keywords** Feed rate · C/N ratio · Percent conversion · Biomass, *D. annae*

## Introduction

Vermicomposting is affected by a number of biotic and abiotic factors including earthworm species, C/N ratio, and feed rate (Lim et al. 2016; Wu et al. 2014). Several epigeic species (*Eisenia fetida*, *Eudrilus eugeniae*, *and Perionyx excavatus*) have been categorized as highly suitable for vermicomposting due to their high reproductive rate, rapid vermiconversion, and tolerance for a wide variety of organic wastes (Sinha et al. 2002; Sharma et al. 2005). These known species have been researched extensively for their biology and waste bioremediation capabilities (Dominguez and Edwards 2011; Pandit et al. 2012; Goswami et al. 2016). However, studies on other potential tropical species are very limited. Rajpal et al. (2013) reported similar performances for *Perionyx sansibaricus* relative to *P. excavatus*, while Suthar and Singh (2007) showed similar performances between *E. andrei* and *E. fetida*. For those species, variations in growth rate, fecundity, and juvenile development have been linked in part to the C/N ratio of the substrate (Suthar and Singh 2007).

While C/N ratio is not sufficient to describe substrate quality, it is often used as the basis for combining substrates (Ravindran and Mnkeni 2016). Having the right balance between C and N is critical to ensuring a favorable environment for earthworm growth and reproduction (Pattnaik and Reddy 2010). Optimal C/N ratio ensures adequate available energy for the conversion of compounds within the matrix of substrate materials (Parthasarathi et al. 2016). Ndegwa and

Responsible editor: Philippe Garrigues

✉ Micah Martin
martinmmicah@gmail.com

[1] Department of Food Production, Faculty of Food and Agriculture, The University of the West Indies, St. Augustine, Trinidad and Tobago

Thompson (2000) suggested that C/N ratios of approximately 25 are best suited for optimizing the interaction between earthworms and microbes during vermicomposting.

Few studies have investigated vermicomposting at C/N ratios outside the previously identified optimal range. As a result, several potential "vermicompostable" substrates and their combinations may not be considered due to unfavorable C/N ratios. Nattudurai et al. (2014) observed a significant ability of *E. eugeniae* to vermicompost high C/N waste (180 to ~30). Lim et al. (2015) also reported that the C/N ratio of empty fruit bunches was reduced from 148.96 to 35.40 in 12 weeks via vermicomposting using *E. eugeniae*.

C/N ratio also affects cocoon production, juvenile development, and adult biomass. Aira et al. (2006) studied *E. fetida* under two C/N ratios and reported higher earthworm biomass, earthworm adult, and juvenile population at a C/N ratio of 19 compared to 11. However, mean adult weight was higher at the lower C/N ratio. It can be inferred from their study that substrates high in nitrogen might increase individual worm biomass, while the opposite fosters increased fecundity. Ndegwa and Thompson (2000) observed a reduction in earthworm biomass with increasing C/N ratios (10, 15, 20, and 25) for *E. fetida*, which also supports the findings of Aira et al. (2006). More in-depth comparative studies are needed to further elucidate species response to C/N ratio as evidence shows variation among species (Makhija et al. 2011).

Edwards et al. (2011) emphasized the need for having proper earthworms to substrate ratio, as this ensures efficient vermiconversion. Having excess substrates in relation to earthworms could change the micro-environmental conditions to anaerobic or lead to thermophyllic breakdown, while the opposite condition could result in competition for food and abiotic resources. Edwards et al. (2011) therefore suggested a ratio of 1:1 (feed/worm) to ensure that casting materials collected were the result of earthworm activity. Ndegwa et al. (2000) investigated varying stocking densities and feed rates and reported that a stocking density of 1.6 kg earthworms/m$^2$ and feed rate of 0.75 kg feed/1.25 kg earthworms produced the best quality vermicompost. Species response to variations in feed rates has not been substantially investigated.

Research into newer species will further expand the opportunities for vermicomposting where exotic species are not always easily accessible (Bhardwaj and Sharma 2015; Sogbesan and Ugwumba 2006). *Dichogaster annae* has a luminescent green appearance, ~3–5 cm long with a diameter of .2 cm and responds very vigorously to external stimuli. It produces a finer casting compared to larger species such as *E. eugeniae* and *P. excavatus* which improves the esthetics of its vermicompost (James and Guimarae 2010). James and Guimarae (2010) listed *D. annae*, as having the potential for vermicomposting; however, subsequent reports on its use have not been forthcoming. Other studies using a related species *Dichogaster bolaui* and *Dichogaster modiglianii* under laboratory conditions indicate their suitability for vermicomposting (Julka 1993; Bhattacharjee and Chaudhuri 2002).

Vermicompost quality is largely dependent on species and their interactions with the biotic and abiotic environment. This study evaluated *D. annae* as a composting earthworm under varying C/N ratios and feed at rates of 1, 1.25, and 2 g feed (dry wt)/g worm/day rates. There is a dearth of information on combinatory effects of these factors on earthworm performance, noting that such factors do not act independently in natural systems. A secondary objective was to compare the performance (vermiconversion, vermicompost quality, earthworm biomass) of *D. annae* against recognized species *E. eugeniae* and *P. excavatus*.

## Materials and methods

### Experiment design

The study was conducted at the Soil Science Greenhouse, UWI, St. Augustine, Trinidad and Tobago. Three earthworm species, *P. excavatus*, *E. eugeniae*, and *D. annae* collected from cattle manure-based vermibeds were subjected to feedstock substrates combined at C/N ratios of 28, 36, and 53. They were fed at rates of 1, 1.25, and 2 g feed (dry wt)/g worm/day. The trial had a factorial design with three replicates and lasted 8 weeks. An experimental unit consisted of a 0.004-m$^3$ plastic bucket, perforated at the lid and base, containing worm and feedstock at rates specified, not exceeding one half of the container volume. C/N ratios of feedstock substrates were determined following methods outlined in Thompson et al. (2001). Substrate C\N ratio formed the basis for treatment combinations (Table 1).

### Feedstock preparation

Substrate material included shredded paper (SP), lawn clippings (LC), and cattle manure (CM). Shredded office paper was collected from the Department of Food Production, UWI, St. Augustine, while lawn clippings were collected from lawn greens on the UWI compound, consisting mainly of Carpet grass. Fresh cattle manure was collected from the UWI Field Station in storage areas where animals were confined with straw bedding accessible to the cattle. Cattle manure was prepared by air drying for 2 weeks followed by manual crushing using a hand-operated compacter. Lawn clippings were collected and dried under similar conditions. All substrates were sifted to pass a 1.25-cm sieve.

Electric conductivity (EC) and pH were measured using a handheld conductivity RS232C meter [Field Scout EC 110 Meter] and IQ 150 pH meter [Spectrum Technologies Inc.]

**Table 1** Selected chemical properties (total Kjeldahl nitrogen (TKN), total organic carbon (TOC); pH; electric conductivity (EC)) of feedstock (cattle manure (CM), shredded paper (SP), lawn clippings (LC)) and C/N ratio treatments

| Feedstock | Physicochemical properties | | |
|---|---|---|---|
| | C/N | pH | EC (dS/m) |
| CM | 32.2 | 7.1 | 7.91 |
| SP | 1203.6 | 7.68 | 1.03 |
| LC | 19.8 | 5.97 | 5.05 |
| Property | C/N treatments | | |
| | 28 | 36 | 53 |
| TOC (%) | 22.48 | 47.25 | 80.52 |
| TKN (%) | 0.79 | 1.29 | 1.51 |
| pH | 6.7 | 7 | 7.2 |
| EC (dS/m) | 6.3 | 6.9 | 5.76 |
| CM | 0.33[b] | 0.54 | 0.43 |
| SP | 0.17 | 0.23 | 0.43 |
| LC | 0.5 | 0.23 | 0.14 |
| % < 2 mm[a] | 51.1 | 44.9 | 33.5 |

[a] The percentage of material in the pre-composted feedstock that passed a 2-mm sieve

[b] Values represent the mass ratio of each substrate used to produce the desired C/N ratio

respectively at a solid to water ratio of 1:5 (Thompson et al. 2001). Total Kjeldahl nitrogen (TKN) was determined by acid digestion of 0.3 g dried milled manure, grass, and paper, followed by Kjeldahl steam distillation (Bremner 1996). Total organic carbon was tested using Nelson and Sommers (1996) loss on ignition method. C/N ratio was determined on a dry mass basis and feed substrates combined to achieve desired ratios (28, 36, and 53).

### Earthworm inoculation

Ten grams each live weight of adult worms were placed in 0.004 $m^3$ plastic containers and fed weekly, at rates of 1, 1.25, and 2 g feed (dry wt)/g worm/day. This amounted to an average of 28, 12, and 40 worms/kg for *P. excavatus*, *E. eugeniae*, and *D. annae*, respectively. Feedstock moisture content was maintained at approximately 70% for the duration of the study, through periodic wetting and monitoring.

Adult and juvenile earthworm biomass was recorded at the end of the incubation period along with average individual adult and juvenile biomass. Adults and juveniles were distinguished by the presence and absence of a clitellum. Percent change in total biomass was calculated as the ratio of the difference between initial and final biomass. Following earthworm extraction from the bedding material and castings, the remaining materials were air-dried and hand-rolled to gently loosen vermicast and other undigested materials. Hand rolling involved applying gentle pressure using a custom made rolling pin. Percent conversion was determined by passing the crushed material through a 2-mm sieve, similar to Ndegwa and Thompson (2001) and calculated using the equation below. Additionally, vermicast was assessed for pH, EC, and C/N ratio following procedures described previously.

$$\text{Percent Conversion} \left[ 100 \times \frac{Mass\ of\ total\ bedding\ material - Mass\ of\ Sifted\ Bedding\ material > 2mm}{Mass\ of\ total\ bedding\ material} \right]$$

### Statistical analysis

Interactive and main effects of earthworm species, C/N ratio, and feed rate were analyzed using a general linear model (GLM) analysis of variance (ANOVA) following procedures on GenStat 16 statistical software (VSN International Ltd., UK). Significant differences among treatment means were further differentiated using Fisher's protected LSD ($P < 0.05$).

## Results and discussion

### Initial feedstock quality

Chemical properties varied among the C/N ratio treatments (Table 1). TKN, TOC, and pH increased with increasing C/N ratio, while EC fluctuated. A greater proportion of CM relative to other substrates at the lowest C/N ratio of 36 is likely responsible for the higher EC of this treatment. Increasing proportion of SP lowered feedstock EC in addition to reducing the amount of material that passed through a 2-mm sieve. The range of experimental C/N ratios provided different levels of labile C and inorganic N, which would test the resilience and ability of the investigated species. Domínguez et al. (2000) stated that different food diets affect the growth and reproduction of vermicomposting earthworm species and that their growth is limited by available carbon content.

### Factor effects

General linear model analysis of variance revealed that species was the most influential factor affecting measured variables

**Table 2** Analysis of variance for C/N ratio, feed rate (FR), and species and their interactions

| Source of variation | Variate | | | | | | |
|---|---|---|---|---|---|---|---|
| | Vermiconversion | Change in biomass | Avg. adult biomass | Juvenile biomass | pH | EC | C/N ratio |
| Species | *** | *** | *** | *** | *** | *** | *** |
| Feed rate | *** | ** | NS | NS | *** | *** | ** |
| C/N ratio | * | *** | *** | *** | *** | *** | NS |
| Species × FR | *** | *** | ** | NS | *** | * | *** |
| Species × C/N | NS | *** | NS | *** | NS | NS | NS |
| FR × C/N | NS | NS | NS | NS | NS | * | NS |
| Specie × FR × C/N | ** | NS | NS | NS | NS | *** | NS |

*NS* non-significant

*Significance at $P = 0.05$

**Significance at $P = 0.01$

***Significance at $P = 0.001$

(Table 2). C/N ratio as well as feed rate affected some parameters. Notably, vermicast C/N ratio was not affected by feedstock C/N ratio probably indicating that feedstock treatments were within a compostable range and of appropriate quality (Ameta et al. 2016). Higher order interactions were less significant among measured variables. Vermicast EC and vermiconversion were the only variables affected by the three-way interaction among species, feed rate, and C/N ratio. To gain the greatest insight into influential factor effects, the results have been restricted to significant factor main and interactive effects across variables.

## Vermiconversion and vermicast quality

Material with particle sizes < 2 mm accounted for > 70% of the earthworm worked substrate compared to < 50% for the initial feedstock. Similar biomass conversion was reported by Ndegwa and Thompson (2001), and this material was considered earthworm castings (Edwards et al. 2011). Substrate conversion to vermicast was significantly ($P < 0.05$) influenced by increasing feed rate and C/N ratio across the three earthworm species (Table 3). At the lowest C/N ratio, *P. excavatus* showed a significantly lower vermicast content when fed at a rate of 2 g feed (dry wt.)/g worm/day than at lower feed rates. An opposite response was observed for *E. eugeniae*, where the lowest conversion occurred at the median rate, while there was similar vermicast production for *D. annae* across feed rates at the low C/N ratio of 28. Increasing C/N ratio significantly increased vermicast production only for *P. excavatus* at the lower feed rate. At the highest C/N ratio, *P. excavatus* was able to maintain a similar conversion compared to a C/N ratio of 36, while *D. annae* increased conversion with an increase in C/N ratio from 36 to 53. At the highest feed rate, both species showed a significantly lower conversion. The opposite trend was observed for *E. eugeniae*. Ndegwa and Thompson (2000) concluded that feedstock with an initial C/N ratio of 25 would be optimal for vermicomposting, as that ratio is favorable for growth of both earthworms and microbes. Our data suggests that across all species increasing, C/N ratio did not inhibit vermiconversion of feedstock substrates. *P. excavatus* and *D. annae* performed exceptionally well at a C/N ratio of 53 as at lower C/N ratios, which may suggest that these earthworms are more suited to high C substrates (Table 1). Garg et al. (2006) reported that *E. fetida* vermicomposted different animal manures at C/N ratios ranging from 88.9 to 137.1, exceeding the ratios reported herein. Results presented in Table 3 support the position that the total content of C and N in the substrate does not provide a true representation of the

**Table 3** Combinatory effects of C/N ratio, feed rate (FR), and earthworm species on substrate vermiconversion

| Species | FR[a] | C/N ratio | | |
|---|---|---|---|---|
| | | 28 % | 36 % | 53 % |
| EE | 1 | 76.51 | 77.9 | 74.44 |
| | 1.25 | 65.8 | 76.5 | 66.76 |
| | 2 | 76.07 | 79.02 | 79.29 |
| PE | 1 | 78.91 | 84.64 | 81.41 |
| | 1.25 | 81.65 | 82.92 | 83.41 |
| | 2 | 74.31 | 72.97 | 70.35 |
| DA | 1 | 78.64 | 80.74 | 86.39 |
| | 1.25 | 75.68 | 77.91 | 83.07 |
| | 2 | 76.16 | 78.57 | 73.27 |
| *LSD (0.05) = 5.78* | | | | |

*EE E. eugeniae*, *PE P. excavatus*, *DA D. annae*

[a] Feed rate (g feed dry wt./g worm/day)

proportion of available nutrients in the substrate (Ndegwa and Thompson 2000). Further investigation is warranted into the fractions of organic C most influential on earthworm behavior.

Manaig (2016) and Suthar (2007) confirmed the ability of *E. eugeniae* and *P. excavatus* to vermicompost different substrates wastes with varying C/N ratios. *E. eugeniae* and *P. excavatus* showed similar potential to vermicompost feedstock with a range of C/N ratios. Increasing the C/N ratio resulted in significant increases in conversion when the feed rate was maintained similar or close to the mass of worms for the smaller *P. excavatus* and *D. annae* species. At the highest feed rate, vermiconversion was lower for all species except *E. eugeniae*. Ndegwa et al. (2000) showed similarly high vermiconversion for *E. fetida* at a feed rate of 1 and 1.25. Although there was undigested material, the earthworms had a greater biomass at these rates. Although similar stocking densities were used, the larger species showed a greater ability to vermicompost greater amount of feedstock. Riggle and Holmes (1994) stated that worms could consume their own weight in 24 h. However, this is dependent on feed quality.

Vermicast EC varied across combinations of species × feed rate × C/N ratio (Table 4). At the low C/N ratio, increasing feed rate resulted in higher vermicast EC for *P. excavatus* and *D. annae*. Statistically similar differences were seen for *E. eugeniae* across feed rates at a C/N ratio of 28. A similar trend was observed for all species at the higher C/N ratio of 36. However, opposite trends were notable at the highest C/N ratio. Increasing feed rate resulted in increasing, fluctuating, and decreasing EC values for *E. eugeniae*, *P. excavatus*, and *D. annae*, respectively. For all species across all feed rates, increasing C/N ratio resulted in decreasing vermicast EC. That was expected as increasing C/N ratio was related to higher amounts of SP (Table 1) which created a dilution effect. Vermicast EC decreased from initial levels for all combinations, with *D. annae* treatments showing the greatest change. Garg et al. (2006) reported similar reductions in EC of different feeds after vermicomposting. However, this is the first report of significant differences among species for this effect. This has practical implications for vermicompost use as soluble salt-derived EC poses a major phytotoxic constraint (Thompson et al. 2001). The results further infer that *D. annae* may have a greater tolerance for high EC feedstock, a claim that is substantiated by the greater fecundity of this species compared to the others at low C/N ratio. Similar claims have also been reported for *E. fetida* (Mupambwa et al. 2016). Garg et al. (2006) suggested that EC > 3.91 dS/m may negatively influence earthworm growth and development.

**Table 4** Combinatory effects of C/N ratio, feed rate (FR), and earthworm species on vermicast EC

| Species | FR[a] | C/N ratio | | |
|---|---|---|---|---|
| | | 28 mS/cm | 36 mS/cm | 53 mS/cm |
| EE | 1 | 4.16 | 4.11 | 3.55 |
| | 1.25 | 4.19 | 3.93 | 3.66 |
| | 2 | 4.15 | 3.96 | 3.90 |
| PE | 1 | 3.92 | 3.78 | 3.86 |
| | 1.25 | 3.95 | 4.08 | 3.34 |
| | 2 | 4.28 | 4.32 | 4.29 |
| DA | 1 | 2.25 | 2.94 | 3.03 |
| | 1.25 | 3.06 | 2.96 | 2.87 |
| | 2 | 3.21 | 3.31 | 2.43 |
| *LSD (0.05) = 0.387* | | | | |

*EE E. eugeniae*, *PE P. excavatus*, *DA D. annae*

[a] Feed rate (g feed dry wt/g worm/day)

Increasing feed per gram of earthworm had nonsignificant effects on final vermicast C/N ratio for *E. eugeniae* and *P. excavatus* (Fig. 1). Substrate vermiconversion by *D. annae* resulted in a significantly higher vermicast C/N ratio when fed at a rate of 1.25. At the highest feed rate, the C/N ratio decreased to 25 but remained significantly higher compared to the other epigeic species. Although these results have been averaged across the different feedstock C/N ratios, *D. annae* seems less capable of waste processing, when greater amounts are present. The greater C/N ratio of *D. annae* vermicast may also support the lower EC values, suggesting lower mineralization despite the feedstock being physically degraded. Similar reductions in C/N ratio were reported by Sharma and Garg (2017). Loss of C as $CO_2$ through microbial respiration and simultaneous addition of N by worms in the form of N excretory materials lowered the C/N ratio (Hussain et al. 2016; Lim et al. 2016. Ravindran and Mnkeni (2016) indicated that a decline in C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic waste. Suthar (2008) using *E. eugeniae* also reported decreases in C/N ratio of vermicompost relative to initial substrate C/N ratio but indicated that the extent of decrease was dependent on the initial C/N ratio. In their study, C/N ratios for some waste exceeded 100. Our research clearly showed that initial feedstock C/N ratio was not influential on final C/N ratio, as both *E. eugeniae* and *P. excavatus* were able to produce vermicast with C/N ratio < 20, irrespective of initial C/N ratio. Further analysis of TKN content of vermicasts showed non-significant differences across C/N ratio treatments, indicating that N retention was not improved at higher C/N ratios. However, a clear species effect was observed. The results suggest that *D. annae* was less effective in stimulating microbial activity at feed rates of 1.25 and 2.

Compared to C/N ratio, vermicast pH showed greater variation between earthworm species and feed rate. Across all

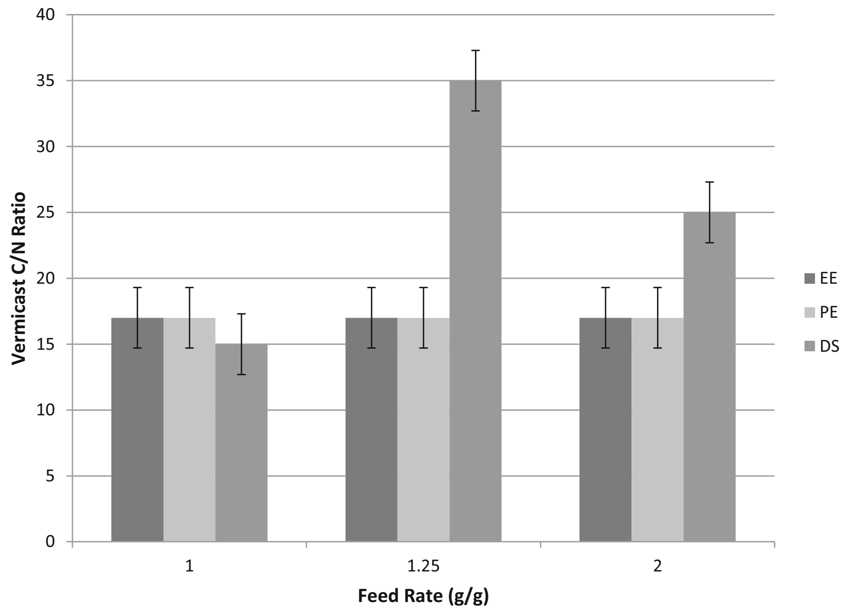


**Fig. 1** Effect of feed rate on vermicast C/N ratio for three epigeic earthworms; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

feed rates, *D. annae* vermicast had significantly higher pH than other treatment combinations (Fig. 2). The highest pH was associated with the lowest feed rate and differed from all other treatments. pH fluctuated for all species with increasing feed rate; however, the increase at 2 g feed (dry wt)/g worm was significant for only *E. eugeniae. P. excavatus* produced the least alkaline vermicast. Relative to the pH of the feedstock, final vermicast pH increased for all treatments, especially for *D. annae*. Atiyeh et al. (2000) and Ndegwa et al. (2000) both reported contrasting trends in pH with a shift towards acidic conditions, associated with the accumulation of organic acid and formation of fulvic and humic acids. Our results support an observation from Datar et al. (1997) who reported a shift in pH towards alkaline conditions increasing with worm density. This comment may corroborate an earlier inference that *D. annae* did not stimulate microbial decomposition, resulting in less mineralization, organic acid production, and other pH reducing compounds. Notable also is the higher biomass increase for *D. annae*, which supports the observations made by Datar et al. (1997).

Increasing C/N ratio > 28 increased vermicast pH, which may be related to increased quantities of C-based substrates relative to N. This may have resulted in lower or different microbial activity reducing N organic compounds, which are associated with lowering pH.

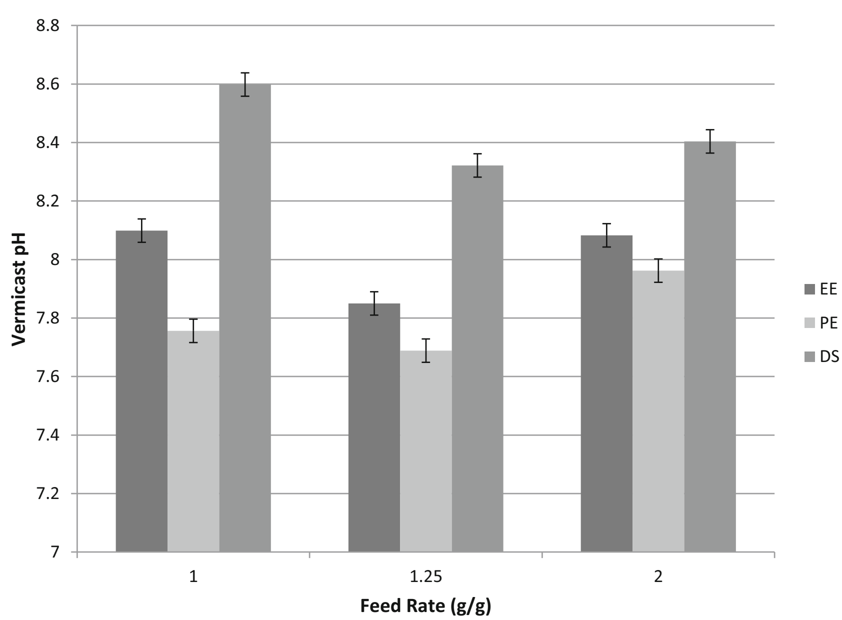


**Fig. 2** Effect of increasing feed rate on vermicast pH for three epigeic earthworms; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

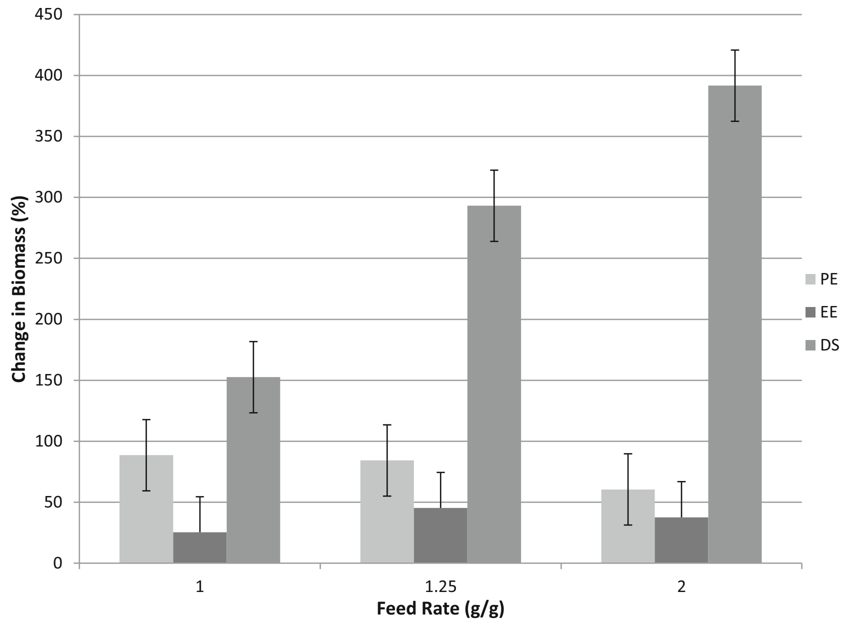


**Fig. 3** Effect of increasing feed rate on change in biomass for three earthworm species; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

## Earthworm biomass

Increasing feed rate resulted in nonsignificant differences for *E. eugeniae* and *P. excavatus*, with the latter maintaining a greater biomass at all feed rates. Contrastingly, change in biomass for *D. annae* increased significantly with increasing feed rate (Fig. 3). Ndegwa et al. (2000) showed similar increases in earthworm biomass of *E. fetida* with increasing feed rate to a maximum rate of 1.25 but did not explain the response. However, they indicated that the response was modified by stocking density. Stocking density varied during composting across species. The average population was 28, 12, and 40 worms/kg for *P. excavatus*, *E. eugeniae*, and *D. annae*, respectively. The smaller earthworm, stocked at the highest density, showed the greatest increase in biomass. Dominguez and Edwards (2011) inferred that higher earthworm biomass at greater stocking density was due to earlier sexual maturity. This is supported by the significantly higher number of adult earthworm observed at all feed rates for *D. annae* (data not shown). *E. eugeniae* and *P. excavatus* have been reported to achieve sexual maturity from as early as 5 weeks (Blakemore 2015; Gupta et al. 2015; Dominguez and Edwards 2011). Further investigation into the life cycle of *D. annae* will explain its reproductive behavior. Earlier reports on related species (*D. modiglianii* and *D. bolaui*) under laboratory conditions report short cocoon incubation time,

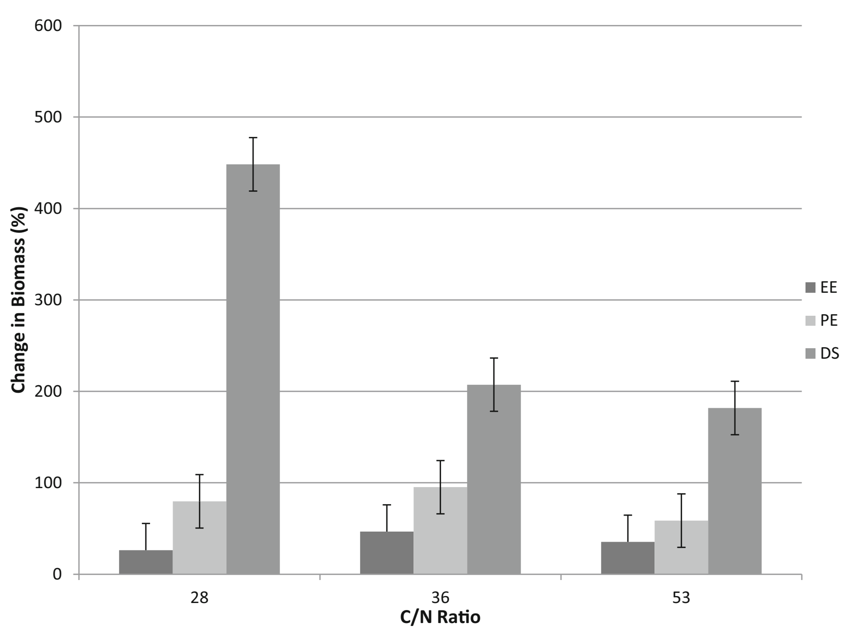


**Fig. 4** Effect of increasing C/N ratio on change in biomass for three earthworm species; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

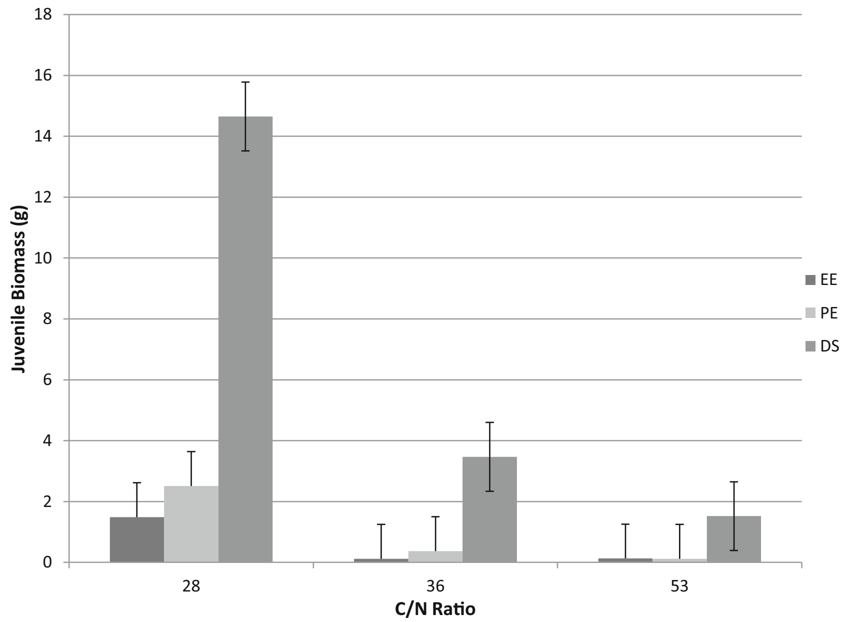


**Fig. 5** Effect of increasing C/N ratio on juvenile biomass for three epigeic earthworms; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

high fecundity, and a 6-week life cycle (Bhattacharjee and Chaudhuri 2002; Julka 1993).

Comparable to increasing feed rate, increasing C/N ratio resulted in nonsignificant changes in earthworm biomass for *E. eugeniae* and *P. excavatus* (Fig. 4). Increasing C/N ratio from 28 to 36 significantly reduced earthworm biomass by > 200% for *D. annae*. Further increase in C/N ratio had no effect on biomass. Results presented for *D. annae* vermicast quality and fecundity support its preference for low C/N ratio feedstock. Ndegwa and Thompson (2000) reported a similar decrease in live earthworm biomass at C/N ratios above 15, although the effect was non-significant for these authors.

Earthworm juvenile biomass (Fig. 5) was affected by species and moderated by feed stock C/N ratio. Juvenile biomass decreased with increasing C/N ratio across all species. The decrease in juvenile biomass was significant for *D. annae* between C/N ratios 28 and 36. At these ratios, *D. annae* also showed significantly higher juvenile biomass compared to *P. excavatus* and *E. eugeniae*. Comparisons between the latter two species were nonsignificant at all C/N ratios. It was hypothesized that the occurrence of a higher stocking density for *D. annae* during vermicomposting may be partly responsible for the increased reproductive activity. Increasing C/N ratio, which resulted in increased vermiconversion, probably affected earthworm fecundity, partitioning energy towards growth.

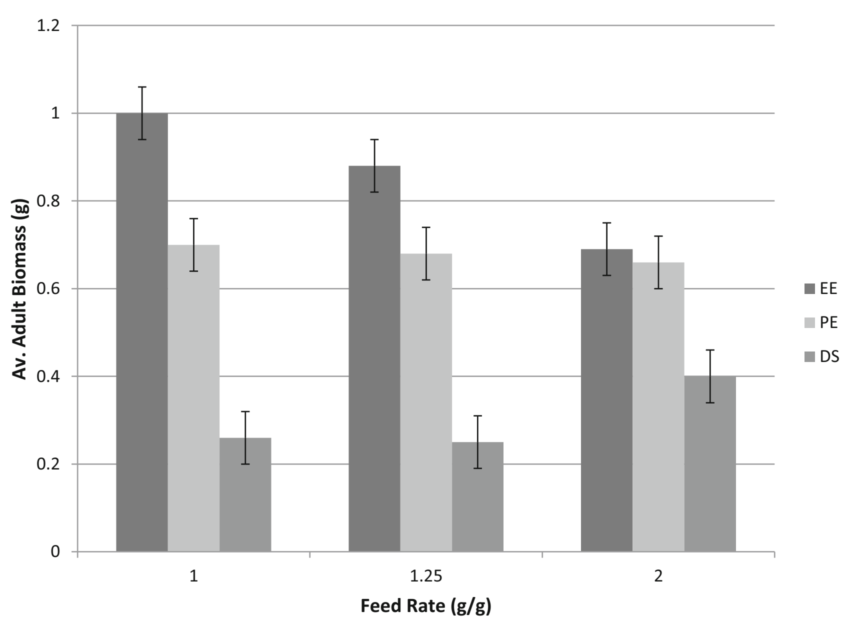


**Fig. 6** Effect of increasing feeding rate on average adult biomass for three epigeic earthworms; *E. eugeniae* (EE), *P. excavatus* (PE), and *Dichogaster* sp. (DS)

Aira et al. (2006) reported contrasting results to that presented herein. They indicated that population structure was affected by C/N ratio with increased juveniles at higher C/N ratios. Notably, in their study, the high C/N ratio was similar to the low C/N ratio in our study, which also showed the greatest juvenile population. The impact of C/N ratio is evident on earthworm population structure with a lower ratio (~25) promoting greater reproduction and juvenile development. Suthar (2008) reported a close negative relationship between cocoon production rate and C/N ratio. Garg et al. (2006) also reported greatest reproductive activity and juvenile biomass in feedstock with low C/N ratio, supporting the position that low C content favors reproduction compared to growth and development.

Increasing feed rate significantly affected average adult biomass of *E. eugeniae* only, with the other species showing similar masses across feed rates (Fig. 6). Interestingly, at the highest feed rate, the average adult biomass was similar for *E. eugeniae* and *P. excavatus*. Suthar (2008) described similar findings. Increasing C/N ratio to ~42 increased mean earthworm weight. Suthar (2008) inferred that the response was related to feed quality, which may be masked within the C/N ratio. Greater attention may be warranted at distinguishing between labile and recalcitrant C of substrates as previously suggested.

## Conclusion

Earthworm vermiconversion of feedstock was >70% for *P. excavatus* and *D. annae* across all feed rates and C/N ratios. Vermicast production decreased with increasing feed rate for *P. excavatus* and *D. annae* with an opposite reaction observed for *E. eugeniae*. Vermicast EC was most affected by C/N treatment, while pH and vermicast C/N ratio by species. *D. annae* produced vermicast with elevated pH and C/N ratio. Adult biomass increased for all species relative to initial values and showed a positive response to increasing feed rate. The differences were most notable for *D. annae*. All species were capable of vermiconverting feedstock as well as partitioning energy towards growth and reproduction at all C/N ratios. However, increasing C/N ratio decreased juvenile population biomass. *D. annae* has shown tremendous potential to vermicompost feedstock ranging in C/N ratio but operates best at C/N ratios >25. For vermicast production across all species, a high C/N ratio and low feed rate were best, while the opposite stimulated reproductive activity and juvenile production.

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