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Impacts of wetting-drying cycles on short-term carbon and nitrogen dynamics in *Amynthas* earthworm casts

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ABSTRACT

Effects of earthworm casts on soil nutrient dynamics and their responses to changing moisture availability in subtropical ecosystems remain poorly understood. This study aimed to examine short-term carbon (C) and nitrogen (N) dynamics and their interactions with wetting-drying cycles in three different structural forms (*i.e.*, granular, globular, and heap-like) of *Amynthas* earthworm casts. The rates of C and N mineralization in the earthworm casts were examined under two different wetting-drying cycles (*i.e.*, 2-d and 4-d wetting intervals) using a rainfall simulation experiment. After three simulated rainfall events, subsamples of the earthworm casts were further incubated for 4 d for the determination of CO_2 and N_2O fluxes. The results of this study indicated that the impacts of wetting-drying cycles on the short-term C and N dynamics were highly variable among the three cast forms, but wetting-drying cycles significantly reduced the cumulative $CO_2$and N_2O fluxes by 62%–83% and 57%–85%, respectively, when compared to the control without being subjected to any rainfall events. The C mineralization rates in different cast forms were affected by the amount of organic substrates and N content in casts, which were associated with the food preference and selection of earthworms. Meanwhile, the cumulative N_2O fluxes did not differ among the three cast forms. Repeated wetting and drying of casts not only enhanced aggregate stability by promoting bonds between the cast particles, but also inhibited microbial survival and growth during the prolonged drying period, which together hindered decomposition and denitrification. Our findings demonstrated that the interactions between the structural forms, aggregate dynamics, and C and N cycling in the earthworm casts were highly complex.

Key Words: aggregate stability, Cand N cycling, earthworm activity, earthworm casting, greenhouse gas flux, mineralization, organic matter, rainfall simulation

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INTRODUCTION

Earthworm activities, such as feeding, casting, and burrowing, are known to influence soil physical properties, nutrient availabilities, and microbial activities. Earthworm casting activities can cause changes to aggregate stability (Shipitalo and Protz, 1988; Blanchart *et al*., 2009) and tensile strength of soils (McKenzie and Dexter, 1987). Earthworms can also alter soil profiles directly by bringing soils from lower horizons to the surface through their burrowing and casting activities (Darwin, 1881). Furthermore, earthworms ingest soil particles, partially break down the organic matter and stimulate microfaunal activity when organic substrates and soil minerals pass through their digestive tracts, and subsequently egest soil aggregates as casts (Edward and Bohlen, 1996; Whalen *et al*., 2004). Since earthworms only ingest particles smaller than the diameter of their intestinal tract and rarely feed on coarse sand with larger particle sizes (Shipitalo and Protz, 1988), their selective ingestion behavior can greatly influence the structure and texture of casts.

Many studies have demonstrated the importance of earthworm surface casts in relation to soil nutrient dynamics in the drilosphere, including the enhancement of nitrogen (N) mineralization and microbial activities during the transit of soil and organic matter through the earthworm gut (Scheu, 1987; Decaëns *et al*., 1999), the promotion of carbon (C) sequestration within soil aggregates (Lavelle and Martin, 1992; Schrader and Zhang, 1997; Bossuyt *et al*., 2005; Jouquet *et al*., 2011; Hedde *et al*., 2013), alteration in the vertical distribution of calcium carbonate granules (Canti and Piearce, 2003), and mobilization of micronutrients in soil (Bityutskii *et al*., 2012). Earthworm casting activities enhance soil aggregate stability, leading to a higher content of organic C especially in the silt- and clay-sized particles (Guggenberger *et al*., 1996). Besides, the higher N mineralization rate in fresh earthworm casts often results in greater available N content in casts than in bulk soil (Scheu, 1987; Decaëns *et al*., 1999). The high mineral N availability in casts can be attributed to the presence of N-containing excretory products and mucus from earthworms, as well as

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the enhanced mineralization of N arising from the intense microbial activity in the earthworm gut (Edward and Bohlen, 1996; Haynes *et al*., 2003). The C:N ratio of earthworm intestinal and cutaneous mucus (*ca*. 6) is usually higher than that of the original resource used, which implies that the excess N assimilated has to be excreted mainly in the form of ammonium and can become incorporated within the casts (Lavelle and Martin, 1992; Schmidt *et al*., 1999). The anaerobic environment in the earthworm guts and casts provides favorable conditions for denitrifying bacteria to grow (Lubbers *et al*., 2013), leading to the higher denitrification rates observed in earthworm casts than in soils (Parkin and Berry, 1994).

In addition to the physical processes and the *in vivo* chemical processes in the earthworm gut, abiotic factors such as rainfall intensity also play a key role in modifying the aggregate stability of casts (Shipitalo and Le Bayon, 2004). In the humid tropics and subtropics, strong rainfall intensity can cause severe soil erosion and soil aggregate loss (Shipitalo and Protz, 1988; Mariani *et al*., 2007). The combined effects of surface runoff and rain splash could disintegrate the earthworm casts when the kinetic energy is greater than the aggregate strength, leading to the loss of organic suspended sediments at a rate of 0.12 kg m^{-2} $year^{-1}$(Nooren *et al*., 1995). Repeated wetting-drying cycles, along with soil texture are also shown to have remarkable influences on the C and N dynamics in stabilized earthworm casts (McInerney and Bolger, 2000). The enhancement of aggregate breakdown following rewetting further exposes the protected soil organic matter to microbial attack, leading to the depletion of stable C pool in soils (Xiang *et al*., 2008). At the same time, soil rewetting can generate a pulse of dissolved organic N, which is susceptible to subsequent mineralization and nitrification, by facilitating the metabolism of bacterial osmolytes (Schimel *et al*., 2007; Xiang *et al*., 2008). The extent of nutrient mineralization during wetting-drying cycles thus depends, at least partly, on the size and quality of organic matter in earthworm casts.

Fresh earthworm casts are less stable than bulk soil (Hindell *et al*., 1997), but their stability can increase after repeated drying and rewetting over time by forming bonds between soil particles during the dry periods (Marinissen and Dexter, 1990). Fungi and moss colonization on the surface casts during the dry period could also play a role in cast stabilization (Le Bayon and Binet, 2001). The composition and structural stability of casts can vary considerably among earthworm species and soil types (Clause *et al*., 2014), leading to possible differences in the effects of wetting-drying cycles on aggregate stability and nutrient release as a function of the structural form and age of casts (Brown *et al*., 2004; Lavelle *et al*., 2016). For example, compact globular casts could help stabilize soil structure, while small granular casts could induce local erosion and soil creep (Decaëns, 2000; Lavelle *et al*., 2016). In addition, the impacts of drying and rewetting of earthworm casts on soil properties are often species-specific. While the globular casts produced by *Amynthas khami* are water-stable and can reduce soil and nutrient loss in the tropical terrestrial ecosystem during rainfall events (Jouquet *et al*., 2012), the granular casts produced by *Metaphire posthuma* have low aggregate stability and can reduce soil roughness and increase soil erosion in response to rain (Jouquet *et al*., 2013). Although rainfall is expected to exert a stronger impact on casts in warm and humid tropical/subtropical regions than in cold and dry temperate regions, the majority of the existing studies have only investigated rainfall impacts on the earthworm casts of temperate lumbricid species such as the anecic *Lumbricus terrestris* and the endogeic *Allolobophora chlorotica*. Hitherto, little is known regarding the rainfall impact on C and N dynamics in earthworm casts in subtropical ecosystems.

The objective of this study was to evaluate the impacts of wetting-drying cycles on C and N dynamics in surface casts produced by earthworms in the Megascolecidae family with three different structural forms, *i.e*., granular, globular, and heap-like. We hypothesize that: i) prolonged drying of casts after rewetting would reduce C and N mineralization rates as compared to controls that are not subjected to any rainfall events, and ii) the effects of wetting-drying cycles on C and N mineralization would vary with aggregate stability and organic matter content in earthworm casts.

MATERIALS AND METHODS

Sampling site description

Soils and earthworm surface casts were sampled at the Tai Po Kau Nature Reserve (22°25′ N, 114°10′ E), one of the most mature secondary woodlands in Hong Kong, China. This reserve lies in the central part of the New Territories at the eastern border of the Tai Mo Shan Country Park, Hong Kong, China, extending over 460 ha and ranging between 100 m and 350 m in altitude. The secondary lowland forest is dominated by *Machilus* spp. and *Psychotria asiatica* (Nicholson, 1996; Corlett, 1999). The mean monthly rainfall at this site ranges from 300 to 450 mm during the wet season (May–September) and from 20 to 100 mm during the dry season (October–April) (Hong Kong Observatory, 2018). The soil at this site is classified as Udic Ferrisol, with a clay loam texture and a low pH value of 4.6. Total organic C (TOC) content, total Kjeldahl N (TKN) content, and C:N ratio in soil are 41 g kg^{-1}, 3 g kg^{-1}, and 15.2, respectively. The NH_4-N and NO_3-N contents in soil are 120.1 mg kg^{-1} and 4.6 mg kg^{-1}, respectively.

Cast characteristics and sampling

Earthworm casts with three different structural forms, namely granular, globular tower-like, and heap-like, were collected by hand-sorting in August 2017 (Fig. 1). According to our field observations, all three casts (*i.e.*, Cast A, Cast B, and Cast C) were produced by either endogeic or anecic *Amynthas* species. Cast A was granular in structure, typically in the form of small pellets that were distributed on the ground surface. Cast B formed a column-like tower structure through the deposition of globular faecal aggregates of clay materials in successive layers (Hong *et al*., 2011). The cast towers were around 10–20 cm in height and about 3–10 cm in diameter. Each of these towers had a hole of 5–10 mm in diameter running through the center. Cast C formed a heap-like structure with very dark and fine soil grains and leaf litter residues on the ground surface. Since the shape of tower casts could change considerably from granular to angular as a result of raindrop impacts (Jouquet *et al*., 2009), only the casts with rounded aggregates without signs of alteration by rain were collected to eliminate the rain effect (Jouquet *et al*., 2009; Hong *et al*., 2011). However, it was too difficult to collect Cast A in good condition in the field owing to its fragility. Instead, we introduced *Amynthas* sp. earthworms into ten cores that were packed with field soils, kept them in an incubator for about 3 weeks, and then collected the surface casts that were produced.

Determination of cast physiochemical properties

The wet-sieving method was used to determine the size distribution of cast aggregates in triplicate. Four grams of oven-dried cast samples were sieved through a series of four sieves at 1 kPa atmospheric pressure for 3 min using a sieve shaker to obtain five aggregate size fractions: $>$ 2 000 µm (large macroaggregates), 500–2 000 µm (medium macroaggregates), 250–500 µm (small macroaggregates), 53–250 µm (microaggregates), and $<$ 53 µm (silt and clay fractions) (Bossuyt *et al*., 2005). About 50 g cast samples were analyzed for their chemical properties. The NH_4-N and NO_3-N contents were determined by flow injection analysis (FIA, FIAstar 5000 Analyzer, FOSS, Denmark) after KCl extraction. The TOC and TKN contents were determined by the Walkley-Black method and the Kjeldahl oxidation method, respectively.

Simulation of wetting-drying cycles

A small-scale rainfall simulator, consisting of a PVC container (0.5 m $\times$ 1 m $\times$ 0.2 m), a water reservoir, a water tank, and a pump, was used in this study to simulate rainfall events. Four sets of rainfall modules, each equipped with nozzles connected to one-way stopcocks, were installed in the PVC container. The falling height of water drops (57 cm) and the impact angle (90° in a still atmosphere) were carefully controlled in order to ensure that the amount of kinetic energy applied to our samples was uniform (Mariani *et al*., 2007). We calibrated the simulator before each rainfall event to a rainfall intensity of 50 $\pm$ 3.0 mm h^{-1} to simulate the heavy rainfall events in subtropical regions. Tap water (pH 6–7, phosphorus $<$ 0.1 mg L^{-1}, and NH_4-N and NO_3-N $<$ 1 mg L^{-1}) was used in the rainfall simulation.

Fig. 1 Earthworms and casts used in this study: Earthworm A (a), Cast A with granular pellet-like structure (b), Earthworm B (c), Cast B with globular tower-like structure (d), Earthworm C (e), and Cast C with heap-like structure and the accumulation of very dark and fine soil grains and leaf litter residues on the ground surface (f).

Before the start of the experiment, about 100 g of cast sample was placed on a 500-μm mesh grid that was secured to the open top of a 5-L bucket. Four replicate cast samples were subjected to each of the two wetting-drying cycles (*i.e.*, 2-d and 4-d wetting intervals). In the 2-d and 4-d cycles, one simulated rainfall event was applied to the cast samples every two and four days, respectively. A total of three rainfall events were simulated in the experiment, each with a duration of 30 min. After each rainfall event, the wetted cast samples were immediately placed in a temperature-controlled incubator at 20 °C and left to dry. Another four replicate cast samples were constantly moistened and kept in a temperature-controlled incubator at 20 °C without being subjected to any rainfall events to serve as control. At the end of the experiment, *ca*. 50 g casts were collected from each replicate sample for chemical analysis.

Cast incubation and gas sampling

After the end of the rainfall simulation experiment, 2 g (fresh weight) casts were collected from each replicate sample, immediately transferred to a 250-mL Duran laboratory bottle, and then sealed with a cap equipped with a gas sampling port. Another four replicate bottles were supplemented with distilled water instead of soils to serve as control. All the bottles were placed in a temperature-controlled incubator at 20 °C for a total of four days. At the beginning (day 0) and after 2 d of incubation (day 2), 20 mL of headspace air sample was collected from each bottle using a 60 mL syringe equipped with a one-way stopcock, and then immediately transferred to a 12 mL pre-evacuated glass vial for subsequent analyses of CO_2 and N_2O concentrations by gas chromatography (7890A, Agilent Technologies, USA). Following gas sampling on day 2, all the bottles were allowed to stand for 30 min with the caps opened, and distilled water was added to the bottles based on the initial fresh weight of casts to minimize the potential biases caused by substrate depletion and cast dehydration (Scheu, 1987; Abail *et al*., 2017). The procedures of cast incubation and gas sampling described above were then repeated between day 2 and day 4. The flux rates of CO_2 and N_2O were determined by the rate of change in gas concentrations in the bottle headspace between the beginning and the end of the 2-d incubation.

Statistical analyses

The non-parametric independent-samples Kruskal-Wallis test (K-W Test) was used to test for significant differences in aggregate composition among the three cast forms. Two-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) *post-hoc* test was used to test for significant differences in cumulative gas fluxes among the cast forms and the wetting-drying cycles. One-way ANOVA with Tukey's HSD *post-hoc* test was conducted to test for significant differences in cumulative gas fluxes and cast chemical properties (TOC, TKN, C:N ratio, and available NH_4-N and NO_3-N) among the three cast forms within each wetting-drying treatment. All data regarding cast chemical properties were log-transformed before ANOVA to satisfy the assumptions of normality and homoscedasticity. Pearson correlation analysis was conducted for each cast form to examine the relationships between cumulative gas fluxes and chemical properties of casts under wetting-drying treatments.

RESULTS

Cast aggregate size

All three cast forms were mainly comprised of medium macroaggregates with a size fraction of 500–2 000 μm. Results of K-W test showed no significant difference in the percentage of soil mass in each aggregate size fraction among the three cast forms (Table I).

Cast chemical properties

Impacts of wetting-drying cycles on the chemical properties of casts were variable (Fig. 2). In general, the wetting-drying cycles had a significant influence on TKN content across the three cast forms. In Cast A and Cast C, the wetting-drying cycles significantly reduced the TKN content and

TABLE I

Aggregate size fractions in the three earthworm cast forms with different structural forms determined by wet sieving

Cast form[a)]	Aggregate size fraction[b)]				
	> 2 mm	500–2 000 μm	250–500 μm	53–250 μm	< 53 μm
			%		
Cast A	18.6 ± 0.31[c)]a[d)]	43.8 ± 2.31a	20.0 ± 1.39a	14.7 ± 0.18a	3.0 ± 0.78a
Cast B	9.0 ± 2.64a	43.2 ± 18.7a	20.3 ± 6.64a	21.4 ± 11.8a	6.1 ± 2.95a
Cast C	16.9 ± 0.45a	59.7 ± 12.3a	15.0 ± 5.32a	8.3 ± 6.75a	0.2 ± 0.73a

a) See Fig. 1 for details of the three forms.
b) Including five fractions: > 2 mm, large macroaggregate; 500–2 000 μm, medium macroaggregate; 250–500 μm, small macroaggregate; 53–250 μm, microaggregate; and < 53 μm, silt and clay fraction.
c) Means ± standard errors ($n = 3$).
d) Means followed by different letters are significantly different within a column according to the non-parametric independent-samples Kruskal-Wallis test.

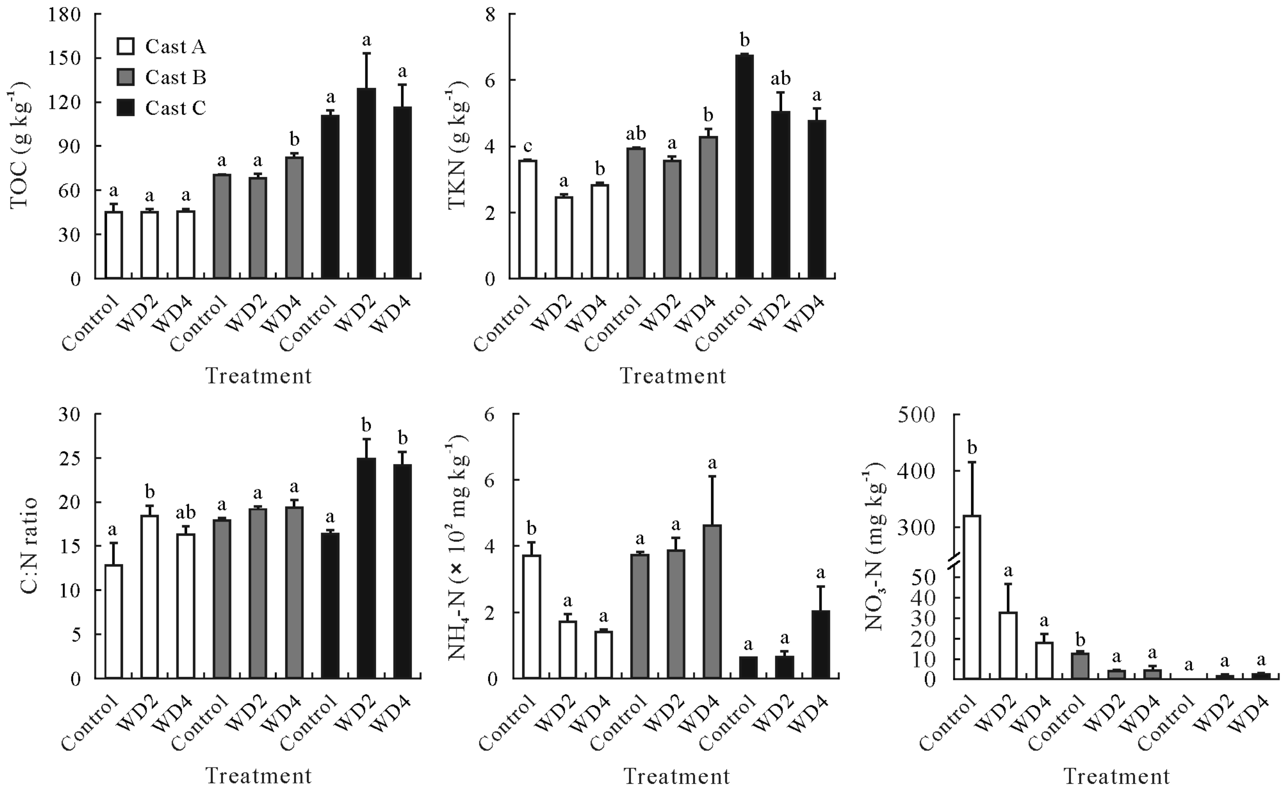

Fig. 2 Chemical composition of the three earthworm cast forms under no wetting (control) or wetting-drying cycles with 2-d (WD2) and 4-d (WD4) wetting intervals. See Fig. 1 for details of the three forms. Values are means and standard errors shown by vertical bars (n = 4). Bars with different letters indicate significant differences among treatments for a given cast form at $P < 0.05$ by analysis of variance with Tukey's honestly significant difference *post-hoc* test. TOC = total organic C; TKN = total Kjeldahl N.

increased the C:N ratio as compared to the control. Moreover, the 2-d and 4-d wetting-drying cycles significantly reduced both the NH_4-N and NO_3-N contents in Cast A, but only decreased the NO_3-N content in Cast B. Meanwhile, the wetting-drying cycles had no significant impacts on both NH_4-N and NO_3-N contents in Cast C.

Cumulative CO_2 and N_2O fluxes

Two-way ANOVA results indicated that the cumulative CO_2 flux was significantly affected by cast forms ($P < 0.01$), wetting-drying cycles ($P < 0.001$), and their interactions ($P < 0.05$). Results of Tukey's HSD *post-hoc* test showed that the cumulative CO_2 fluxes from the control without repeated wetting and drying decreased significantly in the order of Cast C > Cast B > Cast A, while the cumulative CO_2 fluxes from Casts B and C under the 4-d wetting-drying cycle were significantly lower than those of the control (Table II). Moreover, the cumulative N_2O flux was significantly affected by the wetting-drying cycles only ($P < 0.05$), but not cast forms or the interactions between the two factors ($P > 0.05$).

Results of one-way ANOVA showed that the impacts of wetting-drying cycles on greenhouse gas fluxes varied among the casts with different structural forms (Table II). For Cast A, the wetting-drying cycles had no impacts on the cumulative CO_2 emission but significantly influenced the cumulative N_2O flux ($P < 0.05$). The cumulative N_2O flux was the highest from the control (4.2 mg N_2O-N g^{-1} d^{-1}), being significantly higher than that under the 4-d wetting-drying cycle (0.65 mg N_2O-N g^{-1} d^{-1}). For Cast B, the wetting-drying cycles significantly affected the cumulative CO_2 flux ($P < 0.05$) but not the N_2O flux. The cumulative CO_2 flux was the lowest under the 4-d wetting-drying cycle (6.4 g CO_2-C g^{-1} d^{-1}), which was significantly lower than that from the control (29.6 g CO_2-C g^{-1} d^{-1}). For Cast C, both the cumulative CO_2 and N_2O fluxes were significantly influenced by the wetting-drying cycles ($P < 0.05$). The cumulative CO_2 flux from the control (57.6 g CO_2-C g^{-1} d^{-1}) was significantly higher than that under both 2-d and 4-d wetting-drying cycles (9.9 and 15.4 g CO_2-C g^{-1} d^{-1}, respectively). The cumulative N_2O flux was the highest from the control (3.05 mg N_2O-N g^{-1} d^{-1}), which was significantly higher than that under the 2-d wetting-drying cycle (1.02 mg N_2O-N g^{-1} d^{-1}).

Relationships between cast properties and gas fluxes

Results of correlation analysis showed that for Cast A, the cumulative N_2O flux was positively correlated with cumulative CO_2 flux (correlation coefficient r = 0.574, $P <$

TABLE II

Cumulative CO_2 and N_2O fluxes from the three earthworm cast forms under no wetting (control) or wetting-drying cycles with 2-d (WD2) and 4-d (WD4) wetting intervals

Cast form[a)]	Treatment	Cumulative CO_2	Cumulative N_2O
		g CO_2-C g^{-1} d^{-1}	mg N_2O-N g^{-1} d^{-1}
Cast A	WD2	13.2 ± 3.1[b)]aA[c)]	1.13 ± 0.3abA
	WD4	7.5 ± 1.7aAB	0.65 ± 0.3aA
	Control	15.6 ± 1.6aA	4.21 ± 1.2bA
Cast B	WD2	11.3 ± 3.6abA	1.14 ± 0.4aA
	WD4	6.4 ± 1.5aA	1.27 ± 0.4aA
	Control	29.6 ± 6.2bA	1.40 ± 0.4aA
Cast C	WD2	9.9 ± 2.1aA	1.02 ± 0.4aA
	WD4	15.4 ± 1.8aB	1.30 ± 0.2abA
	Control	57.6 ± 6.2bB	3.05 ± 0.1bA

[a)] See Fig. 1 for details of the three forms.
[b)] Means ± standard errors ($n = 4$).
[c)] For a given cast form, different lowercase letters indicate significant differences among treatments within a column, and for a given treatment, different uppercase letters indicate significant differences among cast forms within a column, both at $P < 0.05$ by analysis of variance with Tukey's honestly significant difference *post-hoc* test.

0.05), NH_4-N content (correlation coefficient $r = 0.833$, $P < 0.01$), and NO_3-N content ($r = 0.701$, $P < 0.05$). For Cast B, the cumulative CO_2 flux was positively correlated with NO_3-N content ($r = 0.725$, $P < 0.05$), while the cumulative N_2O flux had no significant relationship with any cast chemical properties. For Cast C, the cumulative CO_2 flux was strongly and negatively correlated with C:N ratio ($r = –0.862$, $P < 0.01$), while the cumulative N_2O flux was positively correlated with both TKN content ($r = 0.687$, $P < 0.05$) and cumulative CO_2 flux ($r = 0.660$, $P < 0.05$).

DISCUSSION

Earthworm cast structure

The composition and structural stability of earthworm casts can be highly variable depending on the species, body shape, size, and casting habits of earthworms, as well as other factors such as soil type, wetting-drying cycles, and the extent of cast aging. Although surface cast production by endogeic earthworms represents only a small proportion of the total volume of egested soils in the tropics, earthworm casting activities are shown to exert considerable influences on the porosity as well as the rates of water infiltration and erosion of tropical soils (Blanchart *et al*., 2004). However, there is currently a paucity of studies examining the impacts of wetting-drying cycles on earthworm casts with different structural forms. Our results demonstrated that in spite of the highly variable structural forms of earthworm casts, ranging from granular with small pellets, globular tower-like, to heap-like with litter residues on the ground surface, the distribution of various aggregate size fractions did not differ significantly among the three cast forms examined in this study.

Carbon mineralization in casts

Our results indicated that cast form was one of the factors governing the variability of cumulative CO_2 fluxes in casts. In the control without the influence of wetting-drying cycles, the cumulative CO_2 flux from the three casts ranged from 15.6 to 57.6 g CO_2-C g^{-1} d^{-1}, with a significantly higher value in Cast C than the other two casts. The difference in C mineralization rates among the cast forms could be related to the variations in organic matter availability in casts arising from substrate selection by earthworms. The mean TOC content in Cast C (110 g kg^{-1}) was considerably higher than that in both Cast A (45 g kg^{-1}) and Cast B (70 g kg^{-1}) (Fig. 2). Earthworms may select food substrates that are rich in organic matter to meet their energy and C requirements, while at the same time increase the TOC content in their casts (Blanchart *et al*., 2004; Abail *et al*., 2017). In our study, we found that both TOC content and cumulative CO_2 flux were the highest in Cast C, which was observed to contain some undigested materials like leaf litter residues. The large amount of organic matter present in Cast C might serve as an important food source for microbes to support a high rate of CO_2 production. Meanwhile, the TOC content might also vary among the casts as a result of a difference in mucus secretion by earthworms or the size distribution of cast particles and aggregates. While the mucus excreted by earthworms might alter the microbial properties of casts (Edward and Bohlen, 1996), the daily loss of C due to mucus excretion in casts was estimated to be only about 0.5% of the total animal C in earthworms (Scheu, 1991). As such, we assumed that the C addition to casts through mucus secretions was not significant, since the influence of mucus secretion on the TOC content of *Amynthas* earthworm casts was largely unknown. Soil particle size is also known to affect C mineralization in casts, as clay particles can bind to phenolic compounds during their passage in the earthworm gut, which would in turn help stabilize the phenolic C in casts (Butenschoen *et al*., 2009). Yet, we found no significant difference in the particle size distribution and the aggregate compositions among the three cast forms. Therefore, it was most likely that substrate selection by different earthworm species was the main cause for the observed variations in TOC content and subsequently the CO_2 flux among the three cast forms.

Nitrogen dynamics in casts

The high contents of inorganic N observed in Casts A and B were in line with the findings of previous studies that earthworm casting activities could increase the content of inorganic N, especially NH_4-N, in casts (Decaëns *et al*., 1999; Haynes *et al*., 2003; Bityutskii *et al*., 2012). This could be due to the preferential ingestion of N-rich plant residues and the excretion of N-containing products by earthworms, as well as the N flush arising from organic matter mineralization in the earthworm gut and fresh casts (Chapuis-Lardy *et al*., 2010). Moreover, earthworm casts are known to be important microsites for denitrification, which can contribute to N_2O fluxes (Elliott *et al*., 1991). We found no significant differences in cumulative N_2O fluxes from casts among the three structural forms, which might imply that cast form had a negligible influence on the denitrification rates in casts. Nevertheless, since the composition and biomass of microbial communities were not quantified in our study, further investigation should be carried out to test this hypothesis.

Effects of wetting-drying cycles on short-term C and N dynamics in casts

Repeated wetting and drying of soil has been shown to cause a decrease in both soil aggregate stability and organic matter content, while at the same time an increase in microbial activity and the mineralization of C and N (Pulleman and Tietema, 1999; Denef *et al*., 2001a, b). This suggests that there is a tight link between aggregate dynamics, soil organic matter decomposition, and nutrient cycling. Although it is relatively well known that earthworms are “soil engineers”, and their casts are important microsites for nutrient cycling, so far little is known regarding the effects of wetting-drying cycles on the structure and chemical properties of earthworm casts with varying characteristics.

Our correlation results showed that the cumulative CO_2 and N_2O fluxes were positively correlated with each other in the granular Cast A and the heap-like Cast C, which implied a strong linkage between the C and N dynamics in casts. In general, the cumulative CO_2 and N_2O emissions from the three casts were lower under the influence of wetting-drying cycles as compared to the control, which was consistent with the previous findings that repeated wetting and drying of casts would suppress organic C decomposition and nitrification (Mikha *et al*., 2005; Xiang *et al*., 2008). However, the impacts of wetting-drying cycles were not uniform among the cast forms (Table II). In the granular Cast A comprised of numerous small faecal pellets, repeated wetting and drying had no effects on the cumulative CO_2 flux, but the 4-d wetting-drying cycle significantly reduced the cumulative N_2O flux by 85% as compared to the control. In contrast, in the globular Cast B, wetting-drying cycles had a significant influence on the cumulative CO_2 flux only, but not N_2O flux. The mean CO_2 flux from Cast B decreased significantly by 78% under the 4-d wetting-drying cycle as compared to the control. In the heap-like Cast C, both the CO_2 and N_2O fluxes were significantly affected by repeated wetting and drying. Compared to the control, the cumulative CO_2 and N_2O fluxes dropped by 83% and 67%, respectively, under the 2-d wetting-drying cycle. Our findings supported the previous idea that the impacts of repeated drying of macroaggregates followed by rewetting on C and N dynamics in casts were highly variable (Jouquet *et al*., 2011, 2013).

Three possible reasons could account for the observed differences in the effects of repeated wetting and drying on CO_2 and N_2O fluxes among the three cast forms. Firstly, the splash effect of rainfall can affect the structural stability of casts, and subsequently the rates of C and N mineralization. During rainfall events, raindrops with kinetic energy greater than the aggregate strength can break cast aggregates into smaller pieces and cause severe cast degradation (Le Bayon and Binet, 2001). Following the fragmentation of aggregates, C that was previously protected within the aggregates can become available to microbes for mineralization (Bossuyt *et al*., 2005). During our rainfall simulation experiment, the granular pellet Cast A degraded and collapsed rapidly after the first rainfall event. This indicated that these granular casts had low soil structural stability, which was in agreement with the findings of Jouquet *et al*. (2013) that granular surface casts produced by tropical endogeic earthworms had low water stability and contributed to soil detachment and erosion. Greater C loss might occur in these fragmented granular casts owing to the reduction in physical protection of C within the microaggregates and macroaggregates in comparison to the globular tower-like and heap-like casts that had a higher structural stability and were more resistant to raindrop impacts (Bottinelli *et al*., 2010). Furthermore, during the simulated rainfall events, the more porous and fragmented casts had greater leaching of inorganic N (NH_4-N and NO_3-N) than the control, resulting in slower rate of N mineralization in casts that were subjected to wetting-drying cycles. Therefore, the structural stability of casts may have a stronger influence on C and N mineralization than wetting-drying cycles, especially when the aggregate stability of earthworm casts is low.

Secondly, the repeated wetting and drying of casts might alter the rates of C and N mineralization by stimulating microbial activities. Although active microbial actions in the fresh earthworm casts can accelerate organic matter decomposition and denitrification, microbial activities tend to decline with time and prolonged drying of casts because of desiccation (Jouquet *et al*., 2011; Abail *et al*., 2017). When the dried casts are rewetted, the bulk movement of water through the casts may help desorb and redistribute the labile C components, which could be available to microbes for conversion into CO_2 gas (McInerney and Bolger, 2000; Xiang *et al*., 2008). Meanwhile, in our study, the drying and rewetting of casts likely inhibited microbial decomposition and denitrification, as both the CO_2 and N_2O fluxes were generally found to be lower in the casts subjected to the wetting-drying cycles. The reduction in microbial activities could be caused by N limitation for microbial growth during the drying period (Mikha *et al*., 2005) and/or diminishing microbial population owing to desiccation during cast drying. Our results of Pearson correlation analysis also showed that the rates of cumulative CO_2 and N_2O fluxes were positively correlated with the N contents in casts regardless of their structural forms. Hence, the total and inorganic N contents in casts could be important variables governing the influence of wetting-drying cycles on greenhouse gas fluxes.

Thirdly, the organic matter dynamics in casts could play a role in shaping the influence of wetting-drying cycles on C transformations. Shipitalo and Protz (1988) reported an increase in cast stabilization by drying and rewetting of casts, which could be associated with the development of fungal hyphae, production of bacterial exopolysaccharides, rearrangement of primary particles, and establishment of clay-polyvalent cation-organic matter linkages with strong organo-mineral bonds (Shipitalo and Protz, 1988; Blanchart *et al*., 2004; Vidal *et al*., 2016). The cumulative CO_2 flux from Cast B was significantly lower under the 4-d wetting-drying cycle than the control, which could be explained by the effects of prolonged air-drying of wetted casts on increasing the release of organic substrates and thus the resistance of cast aggregates to subsequent rewetting (Six *et al*., 2004). We observed no significant differences in cumulative CO_2 flux from Cast C between the 2-d and 4-d wetting-drying cycles, which could be due to the high aggregate stability of this cast in association with its high TOC content and large amounts of litter residues. The rich organic substrates in this cast might require a longer drying duration and a greater number of rainfall events for a more complete mineralization to take place.

CONCLUSIONS

Our rainfall simulation experiment results showed that the C mineralization rates among different structural forms of casts were highly dependent on the amount of organic substrates and N in casts, which were in turn affected by the food preference and selection of earthworms. We observed no significant difference in cumulative N_2O fluxes among the three cast forms, which suggested that the three earthworm species might exert a similar influence on microbe-mediated denitrification. While the effects of wetting-drying cycles on C and N dynamics varied among different structural forms of earthworm casts, repeated wetting and drying of casts would generally suppress CO_2 and N_2O emissions, owing to the enhanced cast aggregate stability as well as reduced microbial survival and growth during prolonged drying periods. The results of this study suggested that the links between cast aggregate dynamics, soil organic matter decomposition, and nutrient cycling were very complex. For instance, wetting-drying cycles had no influence on CO_2 flux from the granular casts with high fragility, owing to the severe physical disturbances caused by raindrops in addition to the low aggregate stability of these casts. For the heap-like casts with high contents of organic substrates, their enhanced water stability could have an overriding influence on C and N cycling in response to drying and rewetting. We also found that the cumulative CO_2 and N_2O fluxes were positively correlated with the N content in casts, leading to variations in the impacts of wetting-drying cycles on greenhouse gas emissions among different cast forms. Further investigations should be done to examine the change in microbial community and biomass in casts under the wetting-drying cycles to better elucidate the role of microorganisms in C and N dynamics in earthworm casts.

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