

Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management

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Received 3 August 2006; received in revised form 1 November 2006; accepted 18 November 2006

Available online 26 December 2006

Abstract

Earthworms are known to be important regulators of soil structure and soil organic matter (SOM) dynamics, however, quantifying their influence on carbon (C) and nitrogen (N) stabilization in agroecosystems remains a pertinent task. We manipulated population densities of the earthworm *Aporrectodea rosea* in three maize-tomato cropping systems [conventional (i.e., mineral fertilizer), organic (i.e., composted manure and legume cover crop), and an intermediate low-input system (i.e., alternating years of legume cover crop and mineral fertilizer)] to examine their influence on C and N incorporation into soil aggregates. Two treatments, no-earthworm versus the addition of five *A. rosea* adults, were established in paired microcosms using electro-shocking. A ¹³C and ¹⁵N labeled cover crop was incorporated into the soil of the organic and low-input systems, while ¹⁵N mineral fertilizer was applied in the conventional system. Soil samples were collected during the growing season and wet-sieved to obtain three aggregate size classes: macroaggregates (>250 μm), microaggregates (53–250 μm) and silt and clay fraction (<53 μm). Macroaggregates were further separated into coarse particulate organic matter (cPOM), microaggregates and the silt and clay fraction. Total C, ¹³C, total N and ¹⁵N were measured for all fractions and the bulk soil. Significant earthworm influences were restricted to the low-input and conventional systems on the final sampling date. In the low-input system, earthworms increased the incorporation of new C into microaggregates within macroaggregates by 35% (2.8 g m⁻² increase; *P* = 0.03), compared to the no-earthworm treatment. Within this same cropping system, earthworms increased new N in the cPOM and the silt and clay fractions within macroaggregates, by 49% (0.21 g m⁻²; *P* < 0.01) and 38% (0.19 g m⁻²; *P* = 0.02), respectively. In the conventional system, earthworms appeared to decrease the incorporation of new N into free microaggregates and macroaggregates by 49% (1.38 g m⁻²; *P* = 0.04) and 41% (0.51 g m⁻²; *P* = 0.057), respectively. These results indicate that earthworms can play an important role in C and N dynamics and that agroecosystem management greatly influences the magnitude and direction of their effect.

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Keywords: Aggregates; Agricultural management; *Aporrectodea rosea*; Earthworms; Soil organic matter

1. Introduction

Soil organic matter (SOM) dynamics and maintenance in agricultural systems have received considerable attention in recent years due to their role both in sustainable agroecosystem functioning and global C dynamics. By contributing greatly to a number of soil properties including soil structure, fertility, porosity, water retention and pH buffering capacity, SOM is fundamental in maintaining fertile and productive soils (Tiessen et al., 1994; Craswell and Lefroy,

2001). Given that soil comprises the largest pool of organic terrestrial C, understanding SOM dynamics is also pertinent to climate change concerns and greenhouse gas mitigation efforts (Cole et al., 1996).

The relationship between SOM and soil aggregate dynamics has been the focus of extensive research for many years (see Six et al. (2004) for a review). Microbial processing of organic matter yields organic binding agents that are critical to the formation and stability of soil aggregates (Tisdall and Oades, 1982). Aggregates in turn, physically protect organic residues by isolating the residues from decomposer organisms and inducing greater anaerobic conditions (Elliott and Coleman, 1988), thus inhibiting

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decay and increasing the residence time of C in soils (Six et al., 2002). Microaggregates (53–250 μm) are particularly important in regulating SOM dynamics due to their high stability and resultant low rates of turnover (Six et al., 2000, 2002). However, by playing a key role in microaggregate formation, macroaggregate (>250 μm) dynamics are also important for C stabilization processes (Elliott, 1986; Six et al., 1998, 2002). Decay of particulate organic matter (POM) within macroaggregates facilitates the adhesion of mineral particles to the organic residues, thus forming stable microaggregates within macroaggregates (Oades, 1984). Microaggregates persist following the breakdown of larger macroaggregates and form a pool of physically protected C with much reduced turnover rates relative to free POM (Six et al., 1998, 2002). While numerous studies have investigated controls on aggregate stability, the role of soil fauna in regulating dynamics of aggregates and associated SOM pools is of particular interest.

As important regulators of decomposition and nutrient dynamics in many ecosystems, earthworm activity can exert considerable influence on SOM dynamics (Lee, 1985; Fragoso et al., 1997; Brown et al., 2000). Furthermore, a number of studies have shown earthworms to greatly influence soil structure, particularly aggregation (Blanchart et al., 1997; Ketterings et al., 1997; Blanchart et al., 1999). As soil and plant residues pass through an earthworm digestive tract they are broken up and thoroughly mixed (Shipitalo and Protz, 1989; Barois et al., 1993). Due to selective foraging of organic particles, gut contents are often enriched in organic matter, nutrients, and water relative to bulk soil and can foster high levels of microbial activity (Haynes and Fraser, 1998; Winsome and McColl, 1998). Upon cast deposition, microbial byproducts, in addition to earthworm mucilages, function to bind soil particles and contribute to the formation of highly stable aggregates (Shipitalo and Protz, 1989). Although earthworms may speed up the initial breakdown of organic residues (Lavelle, 1988; Marinissen and de Ruiter, 1993), several studies have indicated that they may also stabilize SOM through the incorporation and protection of organic matter in their casts (Martin, 1991; Guggenberger et al., 1996; Bossuyt et al., 2004, 2005; Pulleman et al., 2005). For example, Martin (1991) found C to mineralize more rapidly in casts of *Millsonia anomala* than in uningested soil during the first few weeks of an incubation study. However, total C loss was ultimately reduced in casts relative to bulk soil by the end of the 420-day experiment. Visual inspection and quantification of macroaggregate contents by Pulleman et al. (2005) suggested that earthworms can incorporate and stabilize C in soil aggregates. They found earthworm casts in a pasture system to contain large quantities of particulate organic matter (POM) and newly formed microaggregates relative to aggregates formed by physico-genic processes (Pulleman et al., 2005). Using ^{13}C -labeled plant material, Bossuyt et al. (2004) demonstrated organic residues to be rapidly incorporated into micro-

aggregates formed within macroaggregate casts of *Aporrectodea caliginosa* in a laboratory incubation. Further examination of these microaggregates showed them to be highly effective in protecting residue C from decay (Bossuyt et al., 2005). Although these studies provide compelling evidence for the stabilization of SOM by earthworms, further research, especially under field conditions, is needed to assess the magnitude and extent of this influence.

This study aimed to clarify the influence of earthworms on C stabilization and N dynamics within soil aggregates. We expanded upon the incubation studies of Bossuyt et al. (2004) and the inference-based field studies of Pulleman et al. (2005), and sought to directly test the effects of earthworms on C and N dynamics under field conditions. Consequently, this study directly manipulated earthworm population densities in an agricultural setting, and under varying management practices in order to quantify their effect on aggregate and SOM dynamics.

2. Materials and methods

2.1. Site description

This study was conducted at the Center for Integrated Farming Systems (CIFS), University of California, Davis (38°32'N, 122°52'W). The region around the center has a Mediterranean climate with an annual rainfall of 450 mm yr^{-1} . Two soil types, Yolo silt loam (fine-silty, mixed, nonacid, thermic Typic Xerorthent) and Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralf), are found at the site. The experiment was carried out during the 2004 maize growing season, within three irrigated maize-tomato cropping systems under conservation tillage: conventional (only mineral fertilizer), organic (annual winter legume cover crop and composted manure application), and an intermediate low-input system (alternating years of winter legume cover crop and mineral fertilizer). Each management system is replicated by three 0.4 ha plots randomly distributed within a 72 ha field experiment (Denison et al., 2004).

2.2. Experimental design

In March 2004, microplots (1 m \times 1.2 m) were established on pre-existing beds within the replicate field plots for each of the three cropping systems. Microplots included a 2 m buffer on all sides and were managed by hand to simulate management practices in the surrounding field. Maize (*Zea mays* L.) in the conventional system was direct seeded into undisturbed beds following herbicide application in late March. Microplots within this system received two applications of urea mixed with $(^{15}\text{NH}_4)_2\text{SO}_4$ (99 atom%) in solution, yielding a mixture of 6.51 at% ^{15}N . The fertilizer solution was spread evenly over the microplots at a rate of 8.8 g N m^{-2} in early April (NPK starter fertilizer) and 29.3 g N m^{-2} in May (urea). Within

the organic and low-inputs systems, the above-ground portion of the existing cover crop was removed from the microplots and buffer areas shortly before pre-planting tillage operations began in early April. A cover crop (mixture of *Vicia dasycarpa* Ten. and *Pisum sativum* L.) was grown in a greenhouse at UC Davis and labeled with ^{13}C (3.12 atom%) and ^{15}N (7.37 atom%) using methods similar to that described by Bird et al. (2003). In mid-April, after compost application and tillage, the labeled cover crop material was harvested from the greenhouse and incorporated in the low-input and organic microplots at a rate of 300 g dry weight m^{-2} (110 g C m^{-2} and 10 g N m^{-2}) to a depth of 15 cm. Maize was planted in these two cropping systems in early May.

In June of 2004, four microcosms were installed within each of the microplots by inserting PVC cylinders (20 cm dia.) to a depth of 30 cm in the soil. These cylinders were extracted from the ground, maintaining the soil column intact, and sealed on both ends with 1 mm nylon mesh before reinserting them into the microplots. Water was added to bring soil in the microcosms to near field capacity and electro-shocking was used to eliminate pre-existing earthworm populations. This was accomplished by inserting four evenly spaced stainless-steel probes (6.4 mm dia. \times 30 cm deep) along the inside edge of each microcosm and using a portable generator to run an electric current (~ 2 Amps) through the soil for a total of 8 min. The direction of the current was alternated every 2 min between perpendicularly oriented probe pairs for more effective extraction. Five mature individuals of the endogeic earthworm species *Aporrectodea rosea* (~ 1.5 g total fresh wt.) were added to each of two microcosms to establish an earthworm addition treatment (+Worm). Previous observations indicated *A. rosea* to be a common species at this field site and the density of earthworms applied to be well within the range for similarly managed agricultural fields in the region (unpublished data). However, overall earthworm populations in our experimental plots were relatively low and demonstrated high spatial variability. Thus, all *A. rosea* individuals were imported from a nearby farm. To create a zero earthworm treatment (–Worm) the two remaining microcosms were electro-shocked at monthly intervals until the end of the study. All microcosms were monitored and watered regularly (similar to the irrigation schedule for the rest of the field) in order to sustain earthworm activity.

2.3. Field sampling

Soils cores (4.6 cm dia.) were collected to a depth of 15 cm in all microplots, at four sampling times during the course of the experiment: (1) in March, prior to the start of field operations and isotope additions, (2) in June, immediately before establishing earthworm treatments, (3) in early August and (4) in late September before harvest. For both the August and September sampling dates, soil cores were removed from one of the two microcosms in both worm treatments.

2.4. Earthworm recovery

Immediately following the removal of the soil cores, microcosms were excavated and the remaining soil within the microcosms was hand sorted to assess earthworm survival and activity. Earthworms were returned to the lab, dissected, and thoroughly rinsed in order to remove soil from the gut and epidermal tissues. Cleaned earthworm tissues were freeze-dried at -80°C for subsequent analysis.

2.5. Soil fractionation

Soil cores were weighed and sub-samples were removed for determination of moisture content and bulk density. The field-moist soils were gently broken along natural planes of weakness and passed through an 8 mm sieve before air-drying. Sub-samples (80 g) of the air-dried soils were wet-sieved following methods described by Elliott (1986), generating three aggregate size classes: macroaggregates ($> 250\ \mu\text{m}$), microaggregates (53–250 μm) and silt and clay fraction ($< 53\ \mu\text{m}$). Briefly, soil was placed on a 250 μm sieve and slaked in deionized water for 5 min before sieving. The sieve was gently moved up and down by hand for a total of 50 cycles over a 2 min period. Material remaining on the sieve was rinsed into pre-weighed aluminum pans, while material passing through the 250 μm mesh was transferred to a 53 μm sieve for further fractionation. The three size fractions obtained were oven-dried at 60°C . The percentage of soil in the macroaggregate fraction was used as an indicator of aggregate stability.

2.6. Macroaggregate separation

The macroaggregate fraction was separated according to Six et al. (2000). Briefly, 10 g oven-dried macroaggregates were slaked in deionized water for 5 min. These samples were gently shaken with 50 stainless-steel bearings (4 mm dia.) while submerged on top of a 250 μm mesh screen until all macroaggregates were broken. A continuous stream of water flushed the $< 250\ \mu\text{m}$ material through the mesh in order to avoid the break up of microaggregates released from the macroaggregates. Further sieving of the $< 250\ \mu\text{m}$ fraction through a 53 μm sieve, resulted in three size fractions isolated from the macroaggregates: coarse POM ($> 250\ \mu\text{m}$), microaggregates (53–250 μm) and silt and clay ($< 53\ \mu\text{m}$). Each of these fractions was rinsed into pre-weighed aluminum pans and oven-dried at 60°C .

2.7. Carbon, nitrogen and isotope analyses

Sub-samples from each of the free aggregate fractions, the fractions isolated from macroaggregates, along with freeze-dried earthworm tissue were analyzed for total C, ^{13}C , total N and ^{15}N at the UC Davis Stable Isotope Facility using a PDZ Europa Integra C–N isotope ratio mass spectrometer (Integra, Germany).

The incorporation of new C and N into aggregate fractions and earthworm tissues was determined by calculating the proportion, f , of fertilizer (inorganic or cover crop) derived C and N in each fraction using

$$f = (\text{atom}\%_{\text{sample}} - \text{atom}\%_{\text{n.a.}}) / (\text{atom}\%_{\text{label}} - \text{atom}\%_{\text{n.a.}}),$$

where $\text{atom}\%_{\text{sample}}$ is the $\text{atom}\%$ of the sampled material, $\text{atom}\%_{\text{n.a.}}$ is the natural abundance of the element (determined prior to isotope additions) and $\text{atom}\%_{\text{label}}$ is the $\text{atom}\%$ of the labeled material applied. This value was used to calculate the amount of new C or N incorporated within each fraction on a per m^2 basis.

2.8. Statistical analyses

Differences in C and N incorporation into each aggregate size fraction, as well as aggregate stability, were compared independently for each sampling date and across all cropping systems and earthworm treatments using a mixed model ANOVA approach to a randomized split-plot design (with cropping system and earthworm treatments as the main and sub-plot factors, respectively). The model included cropping system, earthworm treatment, and the cropping system by earthworm interaction as fixed variables and field plot as a random variable. Natural log transformations were applied as needed to meet assumptions of ANOVA. In addition, the influence of earthworms was assessed separately for each cropping system and sampling date using paired t -tests to compare the difference in C and N incorporation between the +Worm and –Worm treatments within each plot. Assimilation of C and N into earthworm tissues, within the +Worm treatment, was also compared across cropping systems using ANOVA. All analyses were conducted using JMP IN 5.1 statistical software (SAS Institute Inc).

3. Results

3.1. Influence of cropping system

Cropping system was found to have clear influences on C and N dynamics. Differences between cropping systems are best exemplified by background differences in C and N at the start of the microplot study; the background differences reflect more than 10 yr of differing management systems (Table 1). For example, total C and N in the bulk soil was >50% higher in the organic system than in conventional and low-input systems, and this difference was predominantly explained by differences in macroaggregate and micro-within-macroaggregate associated C and N.

3.2. Aggregate stability

Earthworms had no significant influence on aggregate stability (i.e., proportion of macroaggregates) within any of the cropping systems or at either of the sampling dates.

No significant differences between cropping systems were observed except for the August 2nd sampling data, where the organic system contained 40% more macroaggregates than the low-input and conventional systems ($P = 0.03$).

3.3. Earthworm influence on C and N dynamics

Significant influences of earthworms on C and N incorporation into the soil fractions were observed only for the final (September) sampling date, thus no data for the August sampling date or samplings prior to the earthworm manipulation are reported here. Large and unequal variances in C and N incorporation between cropping systems resulted in a lack of significant differences when examining the earthworm influence across all cropping systems. Much of the observed variability was associated with the organic system. Therefore, further analyses of N incorporation were carried out with the exclusion of the organic system and demonstrated significant cropping system by earthworm interactions for the incorporation of N into macroaggregates ($P = 0.02$), microaggregates ($P = 0.01$) and microaggregates within macroaggregates ($P = 0.048$). This finding of contrasting influences of earthworm activity on N dynamics between the conventional and low-input systems was further confirmed by paired t -tests examining earthworm effects (+Worm vs. –Worm) on aggregate fractions within each cropping system.

Within the low-input system, earthworm activity led to a marginally significant increase in C incorporation into soil macroaggregates by 7.5 g m^{-2} ($P = 0.065$; Fig. 1), indicating a 44% increase in new C incorporation to macroaggregates as compared to the –Worm treatment. This finding is substantiated by a 34% increase of C incorporation into microaggregates within macroaggregates (2.8 g m^{-2} ; $P = 0.03$), as well as apparent increases in both POM and silt and clay associated C within macroaggregates (Fig. 2). Although earthworms appeared to increase N incorporation into all free aggregate fractions within the low-input system (Fig. 3), these effects were not significant ($P > 0.1$). Earthworms did, however, increase N incorporation into the macroaggregate occluded fractions of the low-input system (Fig. 4); coarse POM increased by 49% (0.21 g m^{-2} ; $P = 0.003$) and the silt and clay fraction by 38% (0.19 g m^{-2} ; $P = 0.02$). Nitrogen incorporation into microaggregates within macroaggregates suggested the highest average N increase of the macroaggregate fractions, but was not significant ($P > 0.1$). Within the conventional system, the effects earthworm activity suggested marginally significant decreases in N incorporation (Fig. 3) into macroaggregates by 41% (0.51 g m^{-2} ; $P = 0.057$) and free microaggregates by 49% (1.38 g m^{-2} ; $P = 0.04$). High variability in the organic system resulted in no significant influence of earthworms within this system (Figs. 1–4).

Table 1
Background levels of C and N in aggregate fractions for top 15 cm of soil taken in March 2004 from conventional, low-input and organic cropping systems at the Center for Integrated Farming Systems site (Davis, CA). Standard deviations ($n = 3$) appear below each value in italics

	Conventional (Mg C ha ⁻¹)	Low-input (Mg C ha ⁻¹)	Organic (Mg C ha ⁻¹)	Conventional (kg N ha ⁻¹)	Low-input (kg N ha ⁻¹)	Organic (kg N ha ⁻¹)
<i>Free aggregates</i>						
Macroaggregates (>250 μm)	6.32 <i>1.10</i>	7.50 <i>3.70</i>	12.27 <i>2.45</i>	600.98 <i>80.62</i>	712.52 <i>412.60</i>	1268.85 <i>264.50</i>
Microaggregates (53–250 μm)	6.89 <i>0.86</i>	6.82 <i>0.85</i>	7.80 <i>0.68</i>	687.39 <i>143.49</i>	676.70 <i>74.09</i>	859.94 <i>117.81</i>
Silt and clay <53 μm	3.21 <i>0.50</i>	3.13 <i>0.15</i>	3.56 <i>0.47</i>	317.56 <i>43.81</i>	336.26 <i>21.64</i>	403.64 <i>70.51</i>
<i>Macroaggregate components</i>						
Coarse POM >250 μm	0.32 <i>0.08</i>	0.58 <i>0.21</i>	1.20 <i>0.59</i>	16.20 <i>3.44</i>	31.29 <i>14.10</i>	81.89 <i>37.07</i>
Microaggregates 53–250 μm	3.35 <i>0.85</i>	3.96 <i>2.58</i>	7.16 <i>2.14</i>	268.52 <i>57.10</i>	325.42 <i>218.23</i>	692.75 <i>191.75</i>
Silt and clay <53 μm	2.21 <i>0.43</i>	2.51 <i>1.07</i>	3.82 <i>0.60</i>	187.83 <i>37.35</i>	215.49 <i>94.18</i>	375.11 <i>68.47</i>

POM = Particulate organic matter.

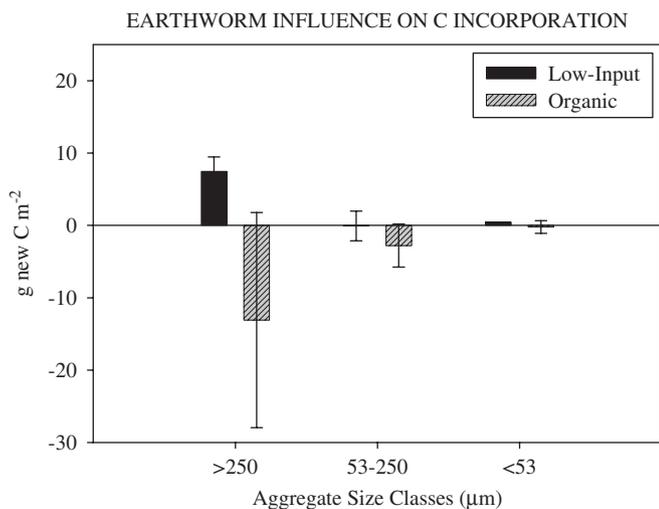


Fig. 1. Earthworm influence on new C (¹³C) incorporation into soil aggregates on the final sampling date. Values represent the average difference between earthworm treatments (plus worm–minus worm). Error bars represent standard errors of the mean.

3.4. Earthworm recovery and C and N incorporation into earthworm tissues

Overall, 78% of the earthworms added to the +Worm microcosms were recovered on the two sampling dates. Although the organic treatment demonstrated slightly lower earthworm recovery, there was no significant difference in the total biomass or the number of earthworms recovered across management treatments or between sampling dates.

N assimilation by earthworms within the conventional system was significantly lower ($P = 0.026$) than in the organic or low-input systems. Despite much greater quantities of ¹⁵N being applied in the conventional system, average ¹⁵N incorporation into earthworms among microcosms within the conventional system was roughly a third

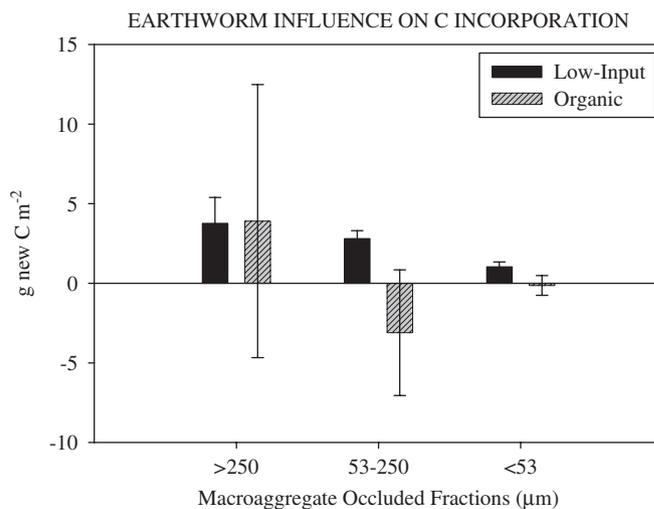


Fig. 2. Earthworm influence on new C (¹³C) incorporation into fractions isolated from macroaggregates on the final sampling date. Values represent the average difference between earthworm treatments (plus worm–minus worm). Error bars represent standard errors of the mean.

of the N incorporation observed in earthworms receiving cover crop-derived ¹⁵N (Fig. 5). No significant differences were found between the organic and low-input systems for either ¹³C or ¹⁵N assimilation into earthworm tissues.

4. Discussion

4.1. Earthworm influences on C storage and aggregate stability

Our findings indicate the potential for the earthworm *A. rosea* to influence SOM stabilization and N dynamics in agroecosystems. Results from the low-input system in this experiment generally corroborate the findings of Bossuyt et al. (2004), in that earthworms increased the concentration of residue-derived C into macroaggregates and the

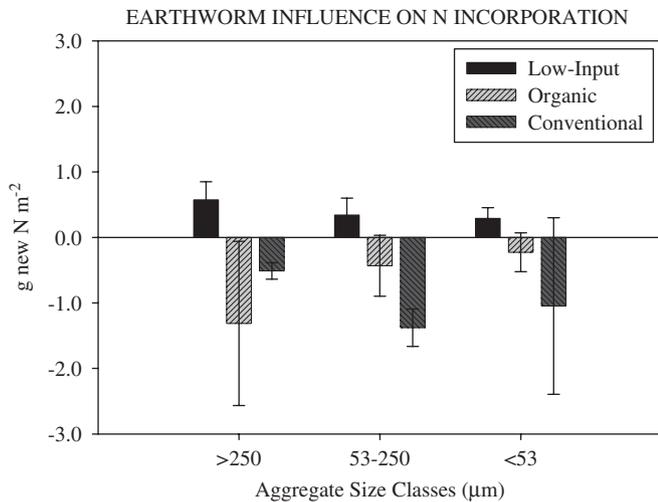


Fig. 3. Earthworm influence on new N (¹⁵N) incorporation into soil aggregates on the final sampling date. Values represent the average difference between earthworm treatments (plus worm–minus worm). Error bars represent standard errors of the mean.

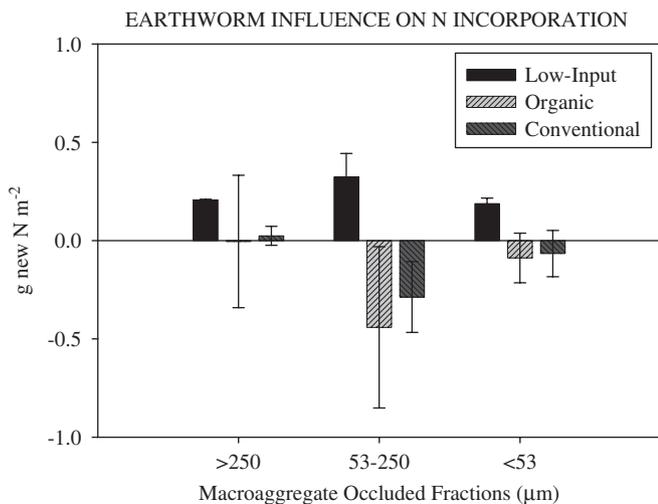


Fig. 4. Earthworm influence on new N (¹⁵N) incorporation into fractions isolated from macroaggregates on the final sampling date. Values represent the average difference between earthworm treatments (plus worm–minus worm). Error bars represent standard errors of the mean.

macroaggregate components (Figs. 1 and 2). By increasing the incorporation of residue C specifically into microaggregates within macroaggregates, both experiments suggest that earthworms may help stabilize SOM via slowing the turnover of newly added C. Several studies have shown this fraction to contribute disproportionately to soil C accumulation following changes in management (Six et al., 2000; Denef et al., 2004; Kong et al., 2005). The findings of Bossuyt et al. (2005) confirm this idea by demonstrating that microaggregates within earthworm casts offer increased protection of newly incorporated C. Despite clear similarities in our findings, earthworm effects in the low-input portion of our experiment are less substantial than those observed in the laboratory study of Bossuyt et al. (2004). This difference likely reflects increased background

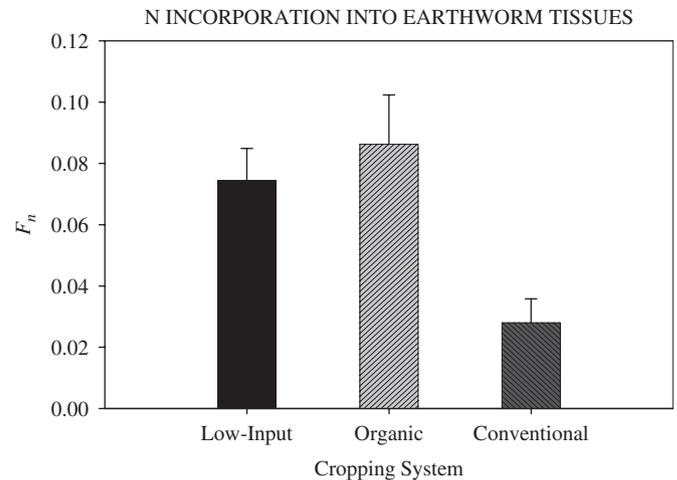


Fig. 5. Fraction (f_n) of earthworm tissue N derived from applied ¹⁵N. Error bars represent standard errors of the mean.

variability that is inherent in field research, along with much lower earthworm densities in our experiment. Furthermore, Bossuyt et al. (2004) found earthworms to have a dramatic effect on aggregate size distribution, greatly increasing the macroaggregate fraction and thus overall C incorporation into macroaggregates, while our study yielded no changes in aggregate size distribution. This apparent discrepancy is likely due to differences in soil disturbance during study preparations. We used intact soils, while the macroaggregate structure was completely eliminated in soil used by Bossuyt et al. (2004). This fits with observations of Villenave et al. (1999) who followed earthworm introductions to several agricultural sites in the Ivory Coast and the Neotropics. They found that beneficial effects of earthworms are most pronounced in highly disturbed soils and may emerge only after decades in soils with more intact structure. Regardless of these differences, both the present study and that of Bossuyt et al. (2004) suggest a potentially important role of earthworms in the stabilization of SOM in agroecosystems.

4.2. N dynamics

Increased incorporation of residue-derived N into soil aggregates within the low-input system generally followed trends observed for C, thus supporting evidence for the earthworm stabilization of SOM. By the final sampling date, both ¹³C and ¹⁵N were elevated in all macroaggregate components (Figs. 2 and 4) within the low-input cropping system, thus indicating the potential for earthworms to facilitate the accumulation of soil N. Although earthworms appear to increase C only in macroaggregates, N was generally elevated for all size fractions within the low-input system (Fig. 3). In accelerating rates of decomposition and N mineralization (Subler et al., 1998; Liu and Zou, 2002), earthworms likely increased N available for incorporation and sorption into other particle size classes, while simultaneously protecting organic N in their casts.

In contrast to trends observed in the low-input system, earthworm activity in the conventional system, where ^{15}N was added as mineral fertilizer, resulted in soil aggregates with decreased ^{15}N contents, indicating a reduced stabilization of N added in this system (Fig. 3). This decrease may be due in part to accelerated denitrification rates within earthworm casts (Elliott et al., 1990; Parkin and Berry, 1999), especially given the highly anaerobic conditions within earthworm casts and increased availability of NH_4^+ and NO_3^- associated with the use of mineral fertilizers. However, earthworms recovered from the microcosms in the conventional system contained much lower ^{15}N concentrations in their tissues than those in the low-input or organic systems (Fig. 5) in spite of the conventional system receiving a three-fold greater addition of ^{15}N . This finding suggests that the decreases we observed for ^{15}N incorporation into aggregates may alternatively result from earthworm avoidance of soil with high concentrations of mineral N. Such behavior would lead to the formation of aggregates that are relatively depleted in N derived from inorganic sources, while the failure of mineral N to become incorporated into aggregates might increase the susceptibility of N loss within this system. Several studies have demonstrated earthworms to facilitate N leaching in agricultural soils (Subler et al., 1997; Dominguez et al., 2004; Wang et al., 2005), suggesting that the same might have occurred in this experiment. Although there is an indication of earthworm induced losses of inorganic N in the conventional system, no significant differences in total ^{15}N recovery between the +Worm and –Worm treatments on the final sampling date suggests that such losses may be small relative to the total N added. We note, however, that even small losses should not be discounted since low-level leaching at greater temporal scales can have significant ecosystem consequences.

4.3. Influence of cropping system

This study highlights the importance of considering management practices in evaluating the influence of earthworms on C and N dynamics. Although differences in C and N dynamics between cropping systems are important, they are not the focus of this paper and thus will not be elaborated upon here except as to their effects on earthworm activity.

Earthworms can have dramatic consequences for nutrient cycling in agroecosystems (Lavelle, 1988; Fragoso et al., 1997), yet their influence may depend greatly on the cropping system in place. The present study indicates an interactive effect of cropping and earthworms on N incorporation into microaggregates. Exclusion of the organic system in our analyses further supports this notion and suggests differential N incorporation into macroaggregates as well as into microaggregates within macroaggregates. Several studies indicate that the earthworm–management interactions observed in this experiment are not entirely unique. For example, Blair et al.

(1997) suggested that earthworms increased soil NO_3^- and NH_4^+ in an agricultural system receiving inorganic fertilizer, but not in systems amended with organic forms of N, such as cover crop or manure. Earthworms, at this same field site, were subsequently found to have a greater effect on N leaching in the system receiving mineral fertilizer, as compared to the systems receiving organic N inputs (Dominguez et al., 2004). Wilcox et al. (2002) compared characteristics of earthworm middens and adjacent soil under a range of management systems, from arable land to forest. In accordance with our findings for soil aggregates, they found middens of *Lumbricus terrestris* to contain higher levels of labile N in all management systems, except for two arable fields receiving high levels of inorganic N. The findings of Pulleman et al. (2005) indicate differential effects of earthworms on soil C depending on management. They found biogenic aggregates (of earthworm origin) to have higher C contents than physicogenic aggregates in both a pasture and in an organic arable system, however, biogenic aggregates from a conventionally managed field were relatively depleted in C. These studies all discuss differential earthworm effects in terms of management and suggest that the form of N applied (mineral vs. organic) drives this difference, as is indicated by our findings. Laboratory studies reporting differential earthworm survival and activity in response to varying N sources (Bohlen et al., 1999; Marhan and Scheu, 2005) further support this suggestion.

Significant results for this experiment were limited almost entirely to the final sampling date and were not present in all cropping systems. We suspect that the density of *A. rosea* applied to the microcosms was insufficient to produce a detectable influence within the 6 wk timeframe examined at the first sampling date. However, we suggest that the sum effects of earthworm activity eventually increased enough to overcome background variability in the low-input and conventional systems by the final sampling date (13 wk). The lack of a consistent influence of earthworms in the organic system leads one to question how the low-input and organic systems, which received identical sources of ^{15}N , could produce such divergent results. We speculate that high background levels of organic C and N already present in the organic system (Table 1), as well as the addition of organic C and N in the form of compost (composted poultry litter at 9Mg ha^{-1} , fresh wt.) shortly before our cover crop application, masked earthworm effects in this study. High variability in ^{13}C and ^{15}N incorporation in the organic system (Figs. 1–4) corroborates this idea and could be explained by minor inconsistencies in compost application or related field operations.

5. Conclusions

We conclude that earthworms have important effects on C and N cycling processes in agroecosystems and that their influence depends greatly on differences in management.

We found earthworms to increase the incorporation of cover crop-derived C into macroaggregates and more importantly into microaggregates formed within macroaggregates. The increased transfer of organic C and N into soil aggregates indicates the potential for earthworms to facilitate SOM stabilization and accumulation in agricultural systems. The influence of earthworms on N cycling, however, appears to be largely determined by cropping system and the form of fertilizer applied (mineral vs. organic). Our results suggest that earthworms have potentially negative consequences on fertilizer-N retention. The findings presented here highlight the potential contribution of earthworms to SOM and nutrient dynamics, and indicate the need to further consider soil fauna in agroecosystem management decisions.

Acknowledgements

We thank Dennis Bryant and the staff at the CIFS field site for their cooperation and assistance in implementing and maintaining our microplot treatments. We are very grateful to Francisco Reis for his extensive assistance in the field and laboratory. We thank Dr. William Horwath and Timothy Doane for use of and assistance with the CO₂ labeling chamber and greenhouse facilities. We thank Dr. Thais Winsome for logistical advice and assistance with earthworm identification. We also appreciate statistical advice provided by Dr. Emilio Laca. Finally, we acknowledge Dr. David Harris at the UC Davis Stable Isotope Facility for technical advice in the use and analyses of stable isotopes. Funding for this project was provided by the Kearney Foundation of Soil Science, the CDFAs Buy California Initiative and the USDA.

References

- Barois, I., Vilemin, G., Lavelle, P., Toutain, F., 1993. Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. *Geoderma* 56, 57–66.
- Bird, J.A., van Kessel, C., Horwath, W.R., 2003. Stabilization of ¹³C-Carbon and immobilization of ¹⁵N-nitrogen from rice straw in humic fractions. *Soil Science Society of America Journal* 67, 806–816.
- Blair, J.M., Parmelee, R.W., Allen, M.F., McCartney, D.A., Stinner, B.R., 1997. Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biology & Biochemistry* 29, 361–367.
- Blanchart, E., Lavelle, P., Braudeau, E., Le Bissonnais, Y., Valentin, C., 1997. Regulation of soil structure by geophagous earthworm activities in humid savannas of Côte D'ivoire. *Soil Biology & Biochemistry* 29, 431–439.
- Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Gilot, C., Pashanasi, B., Lavelle, P., Brussaard, L., 1999. Effects of earthworms on soil structure and physical properties. In: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), *Earthworm Management in Tropical Agroecosystems*. CAB International, Oxon, pp. 149–171.
- Bohlen, P.J., Parmelee, R.W., Allen, M.F., Ketterings, Q.M., 1999. Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates. *Soil Science Society of America Journal* 63, 882–890.
- Bossuyt, H., Six, J., Hendrix, P.F., 2004. Rapid incorporation of fresh residue-derived carbon into newly formed stable microaggregates within earthworm casts. *European Journal of Soil Science* 55, 393–399.
- Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology & Biochemistry* 37, 251–258.
- Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *European Journal of Soil Biology* 36, 177–198.
- Cole, C.V., Cerri, C., Minami, K., Mosier, A., Rosenberg, N., Sauerbeck, D., Dumanski, J., Duxbury, J., Freney, J., Gupta, R., Heinemeyer, O., Kolchugina, T., Lee, J., Paustian, K., Powlson, D., Sampson, N., Tiessen, H., van Noordwijk, M., Zhao, Q., 1996. Agricultural options for mitigation of greenhouse gas emissions. In: Watson, R.T., Zinyowera, M., Moss, R.H. (Eds.), *Climate Change 1995. Impacts, Adaptations, and Mitigation of Climate Change: Scientific-Technical Analyses*. IPCC Working Group II. University Press, Cambridge, UK, pp. 745–771.
- Craswell, E.T., Lefroy, R.D.B., 2001. The role and function of organic matter in tropical soils. *Nutrient Cycling in Agroecosystems* 61, 7–18.
- Denef, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. *Soil Science Society of America Journal* 68, 1935–1944.
- Denison, R.F., Bryant, D.C., Kearney, T.E., 2004. Crop yields over the first nine years of LTRAS, a long-term comparison of field crop systems in a Mediterranean climate. *Field Crops Research* 86, 267–277.
- Dominguez, J., Bohlen, P.J., Parmelee, R.W., 2004. Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. *Ecosystems* 7, 672–685.
- Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil Science Society of America Journal* 50, 627–633.
- Elliott, E.T., Coleman, D.C., 1988. Let the soil work for us. *Ecological Bulletins* 39, 23–32.
- Elliott, P.W., Knight, D., Anderson, J.M., 1990. Denitrification in earthworm casts and soil from pastures under different fertilizer and drainage regimes. *Soil Biology & Biochemistry* 22, 601–605.
- Fragoso, C., Brown, G.G., Patrón, J.C., Blanchart, E., Lavelle, P., Pashanasi, B., Senapati, B., Kumar, T., 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Applied Soil Ecology* 6, 17–35.
- Guggenberger, G., Thomas, R.J., Zech, W., 1996. Soil organic matter within earthworm casts of an anecic-endoic tropical pasture community, Colombia. *Applied Soil Ecology* 3, 263–274.
- Haynes, R.J., Fraser, P.M., 1998. A comparison of aggregate stability and biological activity in earthworm casts and uningested soil as affected by amendment with wheat or lucerne straw. *European Journal of Soil Science* 49, 629–636.
- Ketterings, Q.M., Blair, J.M., Marinissen, J.C.Y., 1997. Effects of earthworms on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. *Soil Biology & Biochemistry* 29, 401–408.
- Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Science Society of America Journal* 69, 1078–1085.
- Lavelle, P., 1988. Earthworm activities and the soil system. *Biology and Fertility of Soils* 6, 237–251.
- Lee, K.E., 1985. *Earthworms: their Ecology and Relationships with Soils and Land Use*. Academic Press, Sydney, 411 pp.
- Liu, Z.G., Zou, X.M., 2002. Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. *Ecological Applications* 12, 1406–1417.
- Marhan, S., Scheu, S., 2005. The influence of mineral and organic fertilizers on the growth of the endoic earthworm *Octolasion tyraeum* (Savigny). *Pedobiologia* 49.

- Marinissen, J.C.Y., de Ruiter, P.C., 1993. Contribution of earthworms to carbon and nitrogen cycling in agro-ecosystems. *Agriculture, Ecosystems and Environment* 47, 59–74.
- Martin, A., 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala* (Omodeo) (Megascolecidae, Oligochaeta) of tropical savannas. *Biology and Fertility of Soils* 11, 234–238.
- Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil* 76, 319–337.
- Parkin, T.B., Berry, E.C., 1999. Microbial nitrogen transformations in earthworm burrows. *Soil Biology & Biochemistry* 31, 1765–1771.
- Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. *Applied Soil Ecology* 29, 1–15.
- Shipitalo, M.J., Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts. *Geoderma* 45, 357–374.
- Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology & Biochemistry* 32, 2099–2103.
- Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal* 62, 1367–1377.
- Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil* 241, 155–176.
- Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research* 79, 7–31.
- Subler, S., Baranski, C.M., Edwards, C.A., 1997. Earthworm additions increased short-term nitrogen availability and leaching in two grain-crop agroecosystems. *Soil Biology & Biochemistry* 29, 413–421.
- Subler, S., Parmelee, R.W., Allen, M.F., 1998. Earthworms and nitrogen mineralization in corn agroecosystems with different nutrient amendments. *Applied Soil Ecology* 9, 295–301.
- Tiessen, H., Cuevas, E., Chacon, P., 1994. The role of soil organic matter in sustaining soil fertility. *Nature* 371, 783–785.
- Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science* 62, 141–163.
- Villenave, C., Charpentier, F., Lavelle, P., Feller, C., Brussaard, L., Pashanasi, B., Barois, I., Albrecht, A., Patrón, J.C., 1999. Effects of earthworms on soil organic matter and nutrient dynamics following earthworm inoculation in field experimental situations. In: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), *Earthworm Management in Tropical Agroecosystems*. CAB International, Oxon, pp. 173–197.
- Wang, X., Hu, F., Li, H.-X., 2005. Contribution of earthworm activity to the infiltration of nitrogen in a wheat agroecosystem. *Biology and Fertility of Soils* 41, 284–287.
- Wilcox, C.S., Dominguez, J., Parmelee, R.W., McCartney, D.A., 2002. Soil carbon and nitrogen dynamics in *Lumbricus terrestris*. L. middens in four arable, a pasture, and a forest ecosystems. *Biology and Fertility of Soils* 36, 26–34.
- Winsome, T., McColl, J.G., 1998. Changes in chemistry and aggregation of a California forest soil worked by the earthworm *Argilophilus papillifer eisen* (Megascolecidae). *Soil Biology & Biochemistry* 30, 1677–1687.