Article published in Ecological Applications (2010) 20:1061-1073

Title: Earthworms and litter management contributions to ecosystem services in a tropical agroforestry system

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Abstract

The development of sustainable agricultural systems depends in part upon improved management of non-crop species to enhance the overall functioning and provision of services by agroecosystems. To address this need, our research examined the role of earthworms and litter management on nutrient dynamics, soil organic matter (SOM) stabilization, and crop growth in the Quesungual agroforestry system of western Honduras. Field mesocosms were established with two earthworm treatments (0 vs. 8 *Pontoscolex corethrurus* individuals per mesocosm) and four litter quality treatments: 1) low quality *Zea mays,* 2) high quality *Diphysa robinioides*, 3) a mixture of low and high quality litters, and 4) a control with no organic residues applied. Mesocosms included a single *Z. mays* plant and additions of ^{15}N labeled inorganic nitrogen. At maize harvest, surface soils (0-15 cm) in the mesocosms were sampled to determine total and available P as well as the distribution of C, N and ^{15}N among different aggregate-associated SOM pools. Maize plants were divided into grain and non-grain components and analyzed for total P, N and ^{15}N. Earthworm additions improved soil structure as demonstrated by a 10% increase in mean weight diameter ($P = 0.024$) and higher C and N storage within large macroaggregates (> 2000 µm; $P < 0.05$). A corresponding 17% increase in C contained in microaggregates within the macroaggregates ($P = 0.033$) indicates that earthworms enhance the stabilization of SOM in these soils; however, this effect only occurred when organic residues were applied. Earthworms also decreased available P ($P < 0.001$) and total soil P ($P = 0.024$), indicating that earthworms may facilitate the loss of labile P added to this system. Earthworms decreased the recovery of fertilizer derived N in the soil ($P < 0.006$), but increased the uptake of ^{15}N by maize by 7% ($P = 0.018$). Litter treatments yielded minimal effects on soil properties and plant growth. Our results indicate that the application of litter inputs and proper management of earthworm populations can have important implications for the provision of ecosystem services (e.g., C sequestration, soil fertility, and plant production) by tropical agroforestry systems.

Keywords: agroforestry, litter quality, nitrogen dynamics, phosphorus, *Pontoscolex corethrurus*, Quesungual, soil structure, soil organic matter, *Zea mays*

Introduction

Agricultural intensification throughout the tropics has reduced the multitude of ecosystem services that agricultural systems have traditionally provided, and has led to the degradation of soils and, therefore, their capacity to support life (Giller et al. 1997, Tilman et al. 2002). In light of this issue, research on sustainable agroecosystem management has placed great emphasis on the efficient use of nutrient resources, the maintenance of soil organic matter (SOM) and the improved management of soil biota in agroecosystems (Sanchez 2002, Barrios 2007). Agroforestry, in particular, has been offered as a valuable means for enabling small farmers in the tropics to supply nutrients for crop growth, while contributing to the long-term fertility and improved biological functioning of soils (Young 1997). In the rural mountains of western Honduras, the Quesungual slash-and-mulch agroforestry system has emerged as a highly successful alternative to traditional slash-and-burn practices that continue to dominate much of the region. In place of burning, this system retains native trees, interspersed with crops, as a source of green manure that is left as mulch on the soil surface (Hellin et al. 1999, Welchez et al. 2008). The lack of burning and continual application of organic inputs not only appears to reduce erosion and improve long-term productivity (Hellin et al. 1999, Rivera Peña 2008), but also promotes soil biological activity through reduced disturbance, improved soil moisture and an expanded detritivorous resource base (Hendrix et al. 1986, Badejo et al. 1995). Earthworms, in particular, appear to benefit from Quesungual management (Pauli 2008) and likely have important, yet poorly quantified impacts on SOM and nutrient dynamics (Lee 1985). Sound management of tropical agroecosystems depends on improved understanding of how alternative management practices (e.g., mulching instead of burning) affect non-target organisms (e.g., soil fauna) and their influence, in turn, on ecosystem functioning and service provision. This study addresses these issues by focusing on the role of earthworms in the Quesungual system and how they interact with the management of organic inputs to influence key agroecosystem services (plant production, soil fertility, SOM stabilization) and ultimately the long-term sustainability of the system.

Often designated as ecosystem engineers (Jones et al. 1994), earthworms are known to influence a number of key soil processes. They can accelerate the decomposition and mineralization of nutrients from organic residues via comminution and incorporation of organic materials into the soil (Lee 1985, Bohlen et al. 1997, Fragoso et al. 1997, Villenave et al. 1999). They also affect these processes indirectly by altering the structure, aeration, and movement of water in soils (Brown et al. 2000). Additionally, earthworms can facilitate the loss of soil nutrients via increased leaching and gaseous losses of N (Parkin and Berry 1999, Dominguez et al. 2004). Despite the potentially beneficial effects on plant growth, the overall influence of earthworms on nutrient cycling and SOM dynamics remains unclear.

Factors regulating the turnover of SOM are of vital concern due to the significant role that organic matter plays in maintaining soil fertility and agroecosystem productivity (Craswell and Lefroy 2001). Furthermore, SOM represents a vast pool of C (Schlesinger 1997) with far reaching implications for global C dynamics and climate change. Research examining the impacts of management on SOM has largely focused on changes to soil structure and the physical stabilization of organic matter within soil aggregates (Paustian et al. 1997, Bronick and Lal 2005). Microaggregates (53-250 µm) are thought to be especially relevant to SOM stabilization due to their high stability and slow turnover time (Angers et al. 1997). Although generally less stable, macroaggregates (>250 µm) often contain higher concentrations of organic matter (Tisdall and Oades 1982) and are thought to provide a site for the formation of new microaggregates (Oades 1984, Golchin et al. 1994, Six et al. 2000). Thus, macroaggregates and the microaggregates formed within them offer a sensitive indicator of management and environmental impacts on SOM dynamics (Six et al. 2002, Denef et al. 2007). Although the formation of microaggregates within macroaggregates has originally been viewed as a microbially-mediated process (Oades, 1984; Golchin et al., 1994), earthworms appear to offer an alternative route for the stabilization of newly added residues in microaggregates within macroaggregates (Bossuyt et al., 2004). Through preferential selection of high C substrates and the complete reorganization of soil structure during gut transit (Shipitalo and Protz 1989, Barois et al. 1993), earthworms appear to rapidly incorporate organic matter into microaggregates formed within their casts (Guggenberger et al. 1996, Bossuyt et al. 2004, Pulleman et al. 2005). Thus, although earthworms have traditionally been thought to accelerate rates of organic matter decay in soils, their influence on soil structure may ultimately lead to the net stabilization of SOM in the long-term (Martin 1991, Brown et al. 2000). Further research is needed to understand the full extent of this phenomenon and understand how earthworms might interact with management to influence C stabilization.

Extensive research has focused on the management of organic resources to improve soil fertility and crop growth (Snapp et al. 1998, Palm et al. 2001). This work has largely examined how litter quality governs the immobilization and release of mineral nutrients and uptake by plants (Palm et al. 2001, Vanlauwe et al. 2005). Although much progress has been made toward improving productivity (Sileshi et al. 2008), the effects of litter additions on soil structure and SOM dynamics is less clear. The addition of organic matter to soil generally improves aggregation and soil C stores (Paustian et al. 1997, Abiven et al. 2009), but the role of litter quality is more ambiguous (Fonte et al. 2009, Gentile et al. 2009). Despite clear effects of litter quality and quantity on earthworm populations (Tian et al. 1997, Barrios et al. 2005, Sileshi and Mafongoya 2007) and well documented impacts of earthworms on SOM and nutrient dynamics, few studies have addressed the potential interactions between litter management and earthworms on soil properties and plant growth.

The research presented here sought to examine the extent to which earthworms and litter additions affect aggregate-associated SOM stabilization, soil nutrient availability, crop growth as well as the movement and uptake of applied inorganic nutrients. We hypothesized that the application of leaf litter and the maintenance of soil cover would promote earthworm populations and lead to greater stabilization of SOM in soil aggregates as well as increased nutrient availability and plant uptake. Additionally, we postulated that quality of litter applied would affect earthworm activity, such that a mix of litter qualities would most benefit earthworm nutrition (Garcia and Fragoso 2003) and yield their greatest impact on soil properties and plant growth.

METHODS

Site description

This study was conducted in the Lempira Department of western Honduras, (N 14°4’, W 88°34’), a mountainous region dominated by a patchwork of cropland, pasture and sub-humid tropical forest. The experiment was installed in May of 2007 on a hillside farm (> 30 % slope) that had been under Quesungual management for 4 yrs since being converted from secondary forest. At 450 m in elevation, precipitation at the site averages 1400 mm yr^{-1} with a distinct dry season between November and May. Mean monthly temperature ranges between 21 and 27 °C.

Soils in this region are generally shallow and rocky, classified as Entisols (Hellin et al. 1999). The field site was characterized by a clay loam texture and a pH of 5.4.

Study design

This experiment employed mesocosms, plastic buckets (18.9 l capacity, 28 cm dia. x 37 cm in depth) with holes (1 mm dia.) drilled in the bottom for drainage, to manipulate earthworm populations and litter additions under field conditions. Each mesocosm was placed in one of 32 pits (40 cm deep) located along three contour rows, with approximately 50 cm spacing between each pit and 80 cm between rows. Soil excavated from the pits was passed through a 12 mm mesh screen to break apart soil clods and remove large rocks and organic materials, then thoroughly mixed. To ensure adequate drainage, the base of each pit was filled with 5 cm of coarse sand before putting the mesocosm in place. The bottom of each mesocosm was then filled with an additional 2 cm of sand before filling the rest of the container with the homogenized soil until level with the surface of the soil outside of the mesocosm (~30 cm deep). Mesocosms were left partially unburied so that a plastic rim (~5 cm tall) remained above the surface of the soil to prevent the entry of upslope materials and to attach a 1.5 mm plastic mesh screen across the top (see Fig 1).

In early June of 2007, after the first rains of the wet season helped to moisten and settle the soil, maize (*Zea Mays;* L.) was seeded into each of the mesocosms. Shortly after emergence, seedlings were thinned to one plant per mesocosm and small holes (5 cm dia.) were cut in the mesh directly above the seedling to permit further growth. One of four litter quality treatments was applied to the soil surface as mulch for each mesocosm. These treatments consisted of: 1) low quality (LQ) dried maize stover from the previous growing season, 2) high quality (HQ) freshly pruned tree leaves (*Diphysa robinioides* Benth., Leguminosae), 3) a mixture of the two litter types (MQ) and 4) a control with no litter added (C). Litter treatments were applied at the time of planting and in early July to simulate mid-season organic inputs associated with normal farmer pruning activities. We attempted to standardize the quantity of organic residues added in each treatment based on the estimated N content in each litter type, so that inputs of C would vary, but added N would not (see Table 1). Inorganic fertilizer was applied to the mesocosms in two applications, according to standard rates in the region, 50 kg N ha^{-1}and 55 kg phosphate ha^{-1} shortly after planting and another 100 kg N ha^{-1} one month later. N was applied as ^{15}N labeled ammonium nitrate (9.9 atom % ^{15}N), while P was added as triple super phosphate. Nutrients were dissolved in water and applied in solution evenly across the soil surface, then watered in to move nutrients to deeper layers and minimize gaseous losses of N. Litter was removed prior to each fertilizer application and replaced immediately afterwards.

Two earthworm treatments, with (+W) and without (–W) worms, were initiated several weeks after maize planting, yielding a total of eight earthworm-litter treatment combinations in a completely randomized design (four replicates per treatment). Earthworms were collected by excavation and hand-sorting of soils adjacent to the study site and returned to the lab for weighing and identification. Eight earthworms (all *Pontoscolex corethrurus*, the dominant species at the field site) were added to the +W mesocosms; this leads to approximate field densities observed in adjacent soils (5.2 g total fresh weight biomass for each mesocosm). Electro-shocking was used at monthly intervals in mesocosms under the –W treatment to keep earthworm colonization (by small juveniles able to pass through drainage holes) at a minimum, while having little direct impacts to soil microbial activity (Staddon et al. 2003). This was achieved using a portable generator and inserting four stainless steel probes vertically (30 cm deep) around the inside edge of each mesocosm. A current (~2 Amps) was then passed through the soil in perpendicular directions by alternating the flow between probe pairs at opposite sides of the mesocosm for a total of 8 minutes.

Soil and plant sampling

Soil cores (two per mesocosm; 2 cm dia. x 15 cm deep) were taken two months after planting (at tasseling of maize) and dried at 45 °C for subsequent analysis of mid-season N availability (potentially mineralizable N). In September 2007, when maize was at physiological maturity, mesocosms were removed from the ground and destructively sampled for all subsequent analyses. Two surface soil cores (9.25 cm dia. x 15 cm deep) were taken from each mesocosm and immediately combined. The field moist surface soils were passed through an 8 mm sieve, by gently breaking soil clods along natural planes of weakness, then dried in at 45 °C prior to laboratory analyses. Soils below 15 cm were sub-sampled, dried, and ground for later analyses.

Maize plants were cut at the soil surface and separated into grain and other above ground components (including stalk, leaves, cob, husk, tassel and silk), while coarse roots (> 2 mm dia.) were recovered from the soil by hand sorting and rinsing with water. All maize components were dried at 60 °C, weighed and then ground to a powder for subsequent analyses.

Earthworm growth, survival and colonization were assessed by hand sorting and thorough inspection of soil in all mesocosms. Earthworms, earthworm pieces and cocoons were counted and weighed in order to determine fresh weight biomass of all earthworm components. A subsample of each large earthworm (those intentionally added to the mesocosms) was dissected and thoroughly cleaned of soil, freeze-dried, and ground in preparation for isotopic analysis.

Soil fertility indices

Potentially mineralizable N was estimated by anaerobic incubation according to adapted methods of Powers (1980). Briefly, subsamples were taken from a composite sample from the two soil cores taken two months after planting. Dry soil (5 g) was submerged in 10 ml of water in a capped centrifuge vial and incubated at 40 °C for 7 days. In order to rapidly induce anaerobic conditions, headspace and dissolved oxygen in the vials was removed by bubbling N_2 gas through the soil solution prior to capping. At the end of the incubation, the soils were shaken with 2 M KCl for 30 min and then centrifuged. Ammonium concentration in the soil extract was then measured colorimetrically (Verdouw et al. 1978) to determine N mineralized during the incubation.

Phosphorus availability in each mesocosm was determined for soils sampled at the end of the growing season in September 2007. Dried soils were sent to the Agriculture and Natural Resources (ANR) Analytical Laboratory (http://groups.ucanr.org/danranlab/) at the University of California, Davis for determination of both Bray and Olsen P. The measurement of Olsen P involved an extraction of bioavailable PO_4 from soil solution using 0.5 M $NaHCO_3$, while the Bray method follows a similar approach, but relies a dilute acid extraction using NH_4F and HCl (Olsen and Sommers 1982).

Aggregate fractionation

Surface soils (0-15 cm) were fractionated by wet-sieving based on the method of Elliott (1986). A subsample (50g) of the dry 8 mm sieved soil from each mesocosm was submerged in deionized water on top of a 2000 µm sieve for slaking. After 5 min. slaking, the sieve was moved up and down in an oscillating motion for 50 cycles over a 2 min period. Aggregates remaining on the sieve were washed into a pre-weighed aluminum pan. Material passing through the sieve was transferred to a 250 µm sieve and sieved in the same manner and again for

2 min. This process was repeated with a 53 µm sieve, yielding a total of four aggregate fractions, large macroaggregates (>2000 µm), small macroaggregates (250-2000 µm), microaggregates (53-250 µm) and silt and clay (<53 µm). The aluminum pans containing water and soil from each size class were then placed in an oven at 60 °C until dried, then weighed to determine the proportion of soil in each fraction. All fractions were ground for subsequent elemental and isotopic analyses. Mean weight diameter (MWD), a measure of aggregate stability, was calculated by summing up the weighted proportions of each aggregate size class following van Bavel (1950).

Large and small macroaggregates were further separated according to Six et al. (2000). Briefly, 6 g of the oven-dried large or small macroaggregates were slaked in deionized water for 20 min then placed on top of a 250 µm modified sieve along with fifty stainless steel ball bearings (4 mm dia.). The soil and bearings were maintained under water and shaken on reciprocal shaker until the bearings had fractured all of the macroaggregates (5-10 min). A continuous flow of water ensured that microaggregates and other materials released from the broken macroaggregates quickly passed through the 250 µm mesh screen to avoid further disruption. This material was then transferred to a 53 µm sieve and sieved for 2 min as described above, yielding a total of three fractions each isolated from large and small macroaggregates: coarse sand and particulate organic matter (>250 µm; cPOM), microaggregates within macroaggregates (53-250 µm; mM) and macroaggregate occluded silt and clay (<53 µm, Msc). These fractions were dried at 60 °C, weighed to determine the contribution of each to large and small macroaggregates, and then ground for subsequent analysis.

Elemental and isotopic analyses

Ground subsamples from the bulk soil (surface and below 15 cm), aggregate fractions, and earthworm tissues were analyzed for total C and N, as well as ^{15}N, while the ground plant components (grain, roots, and other) were analyzed for total N and ^{15}N with a PDZ Europa Integra C–N isotope ratio mass spectrometer (Integra, Germany). Total P in the bulk soils and plant components was measured at the ANR Analytical Laboratory using a nitric acid/hydrogen peroxide microwave digestion and elemental determination by inductively coupled plasma atomic emission spectrometry according to methods of Sah and Miller (1992). The Ash free lignin was determined at the ANR Analytical Laboratory by the reflux method (AOAC 1997).

The proportion of N derived from inorganic N additions in the soil fractions, plant components and earthworm tissues, *f*, was calculated as follows:

$$f = (^{15}\text{N atom\%}_{\text{sample}} - {}^{15}\text{N atom\%}_{\text{n.a.}}) / (^{15}\text{N atom\%}_{\text{source}} - {}^{15}\text{N atom\%}_{\text{n.a.}})$$

where $^{15}\text{N atom\%}_{\text{sample}}$ is the ^{15}N atom% of the sampled material, $^{15}\text{N atom\%}_{\text{n.a.}}$ is the natural abundance of ^{15}N (determined prior to isotope additions) and $^{15}\text{N atom\%}_{\text{source}}$ is the ^{15}N atom% of the applied inorganic N.

Statistical analyses

Comparisons of earthworm and litter influences on mesocosm soil and plant variables, as well as interactions between these two factors, were analyzed using ANOVA. Individual comparisons between litter treatments were carried out using Tukey’s honest significant difference. Orthogonal contrasts were used to specifically examine interactions between earthworms and soil cover (litter treatments versus control), while simple effects of earthworms were evaluated using ANOVA when these interactions proved significant. Multiple linear regression was also used to explore the effect of litter quality (C:N ratio) on potentially mineralizable N in the presence and absence of earthworms. Natural log transformations were applied to the data as necessary to meet the assumptions of ANOVA. All analyses were conducted using JMP 7.0 (SAS Institute 2007).

RESULTS

Treatment effectiveness and earthworm activity

The implementation of earthworm treatments in this study was largely effective. Earthworm survival in the +W mesocosms averaged 80% at the end of the study, with many new juveniles and cocoons observed in this treatment as well. Average ending biomass of the +W treatment was 5.96 g per mesocosm, while earthworms in the –W mesocosms averaged 0.11 g per mesocosm (range = 0.00 to 0.35g). Although the HQ litter treatment demonstrated the highest earthworm survival, final biomass, and cocoon production, there was no significant effect of litter treatment on any of the earthworm components or ^{15}N assimilation by earthworms.

The maize stover applied in the LQ and MQ treatments had a higher N content than was originally estimated (see Table 1). Thus, litter treatments did not differ as greatly in quality as was anticipated and the organic N added in litter was not equal across the LQ, MQ and HQ treatments. Two maize plants (from two different litter treatments) were damaged over the course of the growing season. Although neither of these plants died completely, both plants produced very low above and below ground biomass and these mesocosms were consequently excluded from all analyses.

Soil structure and aggregate associated carbon and nitrogen pools

Earthworm additions significantly increased aggregate stability in surface soils as indicated by a 10% overall increase in MWD (P = 0.024; Fig. 2). Improved soil structure was driven by an increase in large macroaggregates (P = 0.031) and a corresponding decrease in the contribution of free microaggregates (P = 0.048) to the whole soil mass. This influence of earthworms on soil structure translated directly into changes in the distribution of SOM storage within the different aggregate size fractions. Earthworms increased total C storage within large macroaggregates by over 15% (P = 0.048) across all litter treatments. This increase in large macroaggregate SOM was mainly associated with a 17% increase in C contained within the mM fraction (P = 0.033). Overall, microaggregates occluded within both large and small macroaggregates accounted for nearly 60% of total soil C and demonstrated a marginally significant increase in C storage with earthworm additions (P = 0.065; data not shown). Trends for total N contained within each fraction largely mirrored those observed for C, thus data for N is not presented separately.

The maintenance of soil cover appeared to exert control over the effect of earthworms on soil structure and aggregate-associated SOM pools. Orthogonal contrasts (comparing mesocosms with litter applied vs. the control) revealed significant interactions between earthworms and litter application for both MWD (P = 0.005; Fig. 2) and C storage within aggregate fractions. When considering only treatments receiving litter additions, the influence in earthworms becomes more pronounced. Earthworms increased MWD by over 15% in mesocosms receiving litter applications (P < 0.001), but had no effect when the soil was left bare (Fig 2). Similarly, earthworms increased C storage within large macroaggregates and in the associated mM fraction by over 25% (P < 0.005) in the presence of added residues, but had no effect in the control (Figs. 3-4). Litter quality did not yield any significant effects on soil structure or the storage of C or N in any of the aggregate size fractions or bulk soil. Additionally, there were no significant interactions between earthworms and litter type.

Phosphorus and nitrogen availability

Earthworms decreased both Olsen P and Bray P by roughly 30% at the time of harvest ($P < 0.001$). Interestingly, earthworms also decreased total P content of bulk surface soils in the mesocosms by 3.6% ($P = 0.027$; Table 2). Orthogonal contrasts revealed a significant interaction between earthworms and soil cover, such that the effect of earthworms on P availability was greater in microcosms receiving litter applications than in the control ($P = 0.007$ and $P = 0.005$ for Bray and Olsen P; respectively). Litter quality treatments yielded no significant effects on total or available P indices. Potentially mineralizable N was not significantly influenced by either earthworms or litter application; however, mineralizable N did tend to increase with decreasing C to N ratios of residue in the three litter treatments ($P = 0.017$, $R^2 = 0.24$).

Plant growth and nutrient content

Earthworms did not affect the biomass of any of the maize components (Table 3), but there was a marginally significant increase in grain N and P concentration with the addition of earthworms ($P = 0.063$ and P 0.071; respectively). Additionally, earthworms appeared to increase the uptake of P by the non-grain aboveground biomass ($P = 0.001$), mainly due to an increase in the P concentration of this component ($P = 0.029$). The maintenance of soil cover (vs. the control) had no effect on any of the plant components or significant interactions with earthworms. Litter quality however, did produce some effects on plant growth. Total plant biomass, as well as roots and non-grain aboveground maize components increased under the LQ treatment ($P < 0.005$, Table 3). Along with increases in plant biomass, the LQ treatment also increased total N uptake (Table 3) as well as N and P content in the roots and non-grain aboveground biomass ($P < 0.01$). Despite, the various effects on plant growth, there were no significant impacts of earthworms or litter additions on grain yield.

Dynamics of inorganic N additions

Earthworms significantly influenced the redistribution of the ^{15}N applied to each mesocosm. The proportion of fertilizer-derived N, *f*, was decreased by 17% ($P = 0.006$) in bulk surface soils in the presence of earthworms (Fig. 5). Earthworms similarly reduced *f* for all soil aggregate fractions ($P < 0.01$), except for the large macroaggregates (Fig. 5) and components occluded within this fraction (Fig. 6). In fact, earthworms increased the proportion of fertilizer-derived N in the mM fraction of large macroaggregates ($P = 0.050$). The maintenance of soil cover also influenced the movement of ^{15}N within the soil, such that for both free microaggregates and the silt and clay fraction *f* was higher in the control vs. treatments where litter was applied ($P < 0.001$; Fig 5). There was no apparent effect of earthworms or litter quality on the recovery of ^{15}N in soil below 15 cm (Fig 7). However, the application of litter (vs. the control) slightly decreased the amount of ^{15}N recovered in the soil below 15 cm ($P = 0.040$).

In addition to the effects on inorganic N movement in the soil, uptake of N by the maize crop was affected as well. Earthworms increased total ^{15}N recovered in maize plants by 7% ($P = 0.018$, Fig 7), but did not significantly affect the incorporation of fertilizer-derived N into the grain (data not shown). Soil cover, per se, did not appear to affect plant uptake of ^{15}N. Although litter quality did not influence total plant N uptake, the LQ treatment had significantly higher ^{15}N recovery in the roots ($P > 0.001$) and non-grain aboveground components ($P = 0.049$; data not shown). Overall recovery of ^{15}N in the mesocosms (all soil and plant components) averaged 86.8 % and was not significantly influenced by litter quality, soil cover or earthworms ($P > 0.1$).

DISCUSSION

The development of sustainable agroecosystems depends on a better understanding of and reliance upon biological regulation of internal nutrient cycling and related ecosystem processes. The findings presented here contribute towards this goal by offering key insights about the role of soil organisms and litter management in the provisioning of several fundamental ecosystem services in tropical agroforestry systems of Latin America.

Soil structure and aggregate-associated SOM

The influences of earthworms on soil structure observed in this study agree with past findings indicating that earthworms improve aggregate stability (Blanchart et al. 1999, Bossuyt et al. 2004, Shipitalo and Le Bayon 2004, Coq et al. 2007). Although there was no effect of earthworms on total soil C, the redistribution of C into large macroaggregates and specifically the mM fraction (Figs. 3 & 4) suggests that earthworms may enhance C stabilization in these soils. Several studies have demonstrated that earthworms can rapidly incorporate fresh residue C into microaggregates within macroaggregates (Bossuyt et al. 2004, Bossuyt et al. 2006, Fonte et al. 2007). Furthermore, these microaggregates have been shown to contribute greatly to the protection of residue C following the breakdown of the macroaggregates (casts) in which they were formed (Pulleman and Marinissen 2004, Bossuyt et al. 2005). Despite these findings, the influence of earthworms on SOM remains unclear. In a similar experiment to ours, Coq et al. (2007) found *P. corethrurus* to improve aggregation, but to decrease total C in the 10-20 cm layer. They incubated large macroaggregate casts vs. 'non-ingested soil' (20-2000 µm) from the 10-20 cm depth and found higher cumulative C mineralization from the casts after 28 days. Although this result appears to contradict the findings of Pulleman and Marinissen (2004) and Bossuyt et al. (2005), a rapid initial loss of C would be expected following cast formation due to the high concentration of fresh, relatively labile residue (cPOM) in casts. However, given that most of the C in casts (and macroaggregates in general) appears to be associated with the mM fraction (Fig. 4), we suspect that rates of decomposition in casts would eventually decrease below that of the non-ingested soil. In agreement with this idea, Martin (1991) found in a long-term incubation of earthworm casts vs. bulk soil that cumulative C mineralization from casts was lower than that of non-ingested soil after 420 days, despite higher C concentrations and initial rates of CO_2 release in the casts. Several authors have concluded that earthworms facilitate C loss in the short-term, but may stabilize SOM stores over longer time scales and that this effect likely depends on the earthworm species and state of the ecosystem in question (Brown et al. 2000, Lavelle et al. 2004). Although our study did not directly measure the long-term impacts on total soil C, the redistribution of SOM into the mM fraction indicates that *P. corethrurus* helps to stabilize soil C in the long-term within this system.

The maintenance of soil cover (via litter additions) appeared to mediate the effect of earthworms on soil structure and SOM. Earthworms only increased aggregation and SOM stabilization in treatments where litter was added to the mesocosms, suggesting that the effect of earthworms depends on how organic residues are managed. Pulleman et al. (2005) also suggested that the effect of earthworms was dependent on management, such that aggregate stability and C stabilization within casts was greatest in a pasture with high organic matter inputs and low disturbance and lowest in arable fields with low inputs of organic material. Litter quality, per se, did not significantly affect earthworm activity (i.e., growth, cocoon production, ^{15}N assimilation) or their impact on soil properties, thus partially negating our original hypothesis that the quality of applied organic materials would control earthworm activity and thus their effect on soil properties. However, this may be due in part to the smaller than anticipated differences in litter quality parameters (Table 1).

Availability of nitrogen and phosphorus

Although the ability of earthworms to increase N availability in soils is well documented (Pashanasi et al. 1992, Subler et al. 1997, Araujo et al. 2004), this study found no significant effect of earthworms on potentially mineralizable N. The lack of a significant effect likely relates to differences in methodology and the associated pools measured. Most studies have looked directly at mineral N forms, whereas the method employed here measured relatively labile sources of organic N that are likely to become available to plants in the near future (Powers 1980). Although earthworms can increase soil N mineralization in the short-term, this N may be quickly stabilized as casts age (Lavelle et al. 1992, Brown et al. 2000). Thus, the lack of difference in potentially mineralizable N observed in this study may indicate that N released by earthworm activities in the +W treatments does not persist in labile forms following drying. Although litter treatments did not yield significant differences in mineralizable N, litter quality does appear to affect the potential release of N (Constantinides and Fownes 1994, Palm et al. 2001) as was demonstrated by the inverse correlation between potentially mineralizable N and C:N ratio of the litter applied. The relatively small differences between litter quality treatments likely explain the lack of significant differences in ANOVA.

The influence of earthworms on P dynamics was highly significant. However, the observed decrease in soil P availability with the addition of *P. corethrurus* (Table 2) contrasts with numerous studies suggesting that earthworms increase P availability across a wide range of agroecosystems (Sharpely and Syers 1976, Lopez-Hernandez et al. 1993, Jimenez et al. 2003, Kuczak et al. 2006, Le Bayon and Binet 2006). Several possibilities exist to explain this apparent discrepancy. First, nearly all of the studies examining the role of earthworms on soil P dynamics have done so by comparing P in casts vs. non-ingested soil and have largely overlooked the effect of earthworms on the whole soil (cast + non-ingested soil). It is possible that increased P enrichment and availability in casts comes at the expense of lower P content and availability in non-ingested soil. For example, Patrón et al. (1999) found casts of *P. corethrurus* to contain higher levels of total P as well as resin and organic P compared to non-ingested soil, but they found no significant effect of earthworm additions on total P or any of the P fractions in the whole soil. It is also possible that earthworms increased P availability earlier in the growing season, but that this trend had disappeared by the time of measurement (at harvest). Several studies have suggested that increased P availability in casts may be only short-lived (Lopez-Hernandez et al. 1993, Haynes and Fraser 1998, Le Bayon and Binet 2006). Despite the possible explanations for a lack difference in available P, no studies have documented a decrease in soil P availability with earthworm additions.

The lower concentration of total soil P in the +W treatments (Table 2) suggests that earthworms increase the removal of P from the surface layer either through leaching and/or plant uptake. It has been suggested that higher water solubility of P in earthworm casts may facilitate the export of P from agroecosystems (Sharpely and Syers 1976, Le Bayon and Binet 2006). Increased infiltration and leaching associated with earthworm tunneling activities (Subler et al. 1997, Dominguez et al. 2004) may further contribute to P losses and ultimately affect total P in surface layers (Suárez et al. 2003). Given that differences in plant uptake do not explain the loss of P (as was seen for added N), it seems that the export of labile P, presumably maintained in more available state by earthworms earlier in the growing season, resulted in a loss of total P from the surface layer of the worm-worked soils. This would explain how earthworm activity caused a decline in both total and available P pools at the end of the growing season. We should further note that the Entisols used in this experiment were relatively low in total P (approximately 300 kg ha^{-1} in the top 15 cm of soil) and thus annual fertilizer additions likely contribute a large portion of the total P (24.4 kg ha^{-1}, or roughly 8% of the total). Thus, a large proportion of P in these soils appears to exist in a relatively labile form and would be more susceptible to loss than P that is intimately associated with organic matter or mineral surfaces.

Plant growth and yield

The lack of an earthworm effect on plant biomass and yield was unexpected. Earthworms generally have positive effects on plant growth and yield in the tropics, but their influence appears to depend on a number of factors including the crop, earthworm species and soil characteristics of the system being studied (Brown et al. 1999). In their meta-analysis, Brown *et al.* (1999) found that maize is among the crops to best respond to earthworm additions and furthermore, that *P. corethrurus* is a species that often produces large effects. The soil in this study, however, did not fit the criteria for best optimizing the positive influence of earthworms. For example, Brown et al. (1999) found earthworms to have the greatest impact in soils that are low in C content (<1.5%), high in sand (>65%) and intermediate in acidity, while the soils used in this study were intermediate in sand (34%) and C content (2.3%) and slightly more acidic (pH ~ 5.3) than the optimal range. The addition of *P. corethrurus* in this study did seem to increase grain N and P content (suggesting increased nutritional value), but lower (albeit non-significant) average grain yield in the +W treatments effectively nullifies the relevance of this finding. Increases in P content of the non-grain aboveground biomass suggest that earthworms can improve plant P uptake of this highly limiting nutrient. However, the apparent reduction in total soil P urges caution, and emphasizes the need for a more thorough evaluation of earthworm influences on agroecosystem P dynamics.

Although the maintenance of soil cover did not seem to influence plant growth, litter quality did appear to play a small role. Vegetative biomass increased under the LQ treatment, but there was no effect on grain yield (Table 3). Improved growth in the LQ treatment may be due to the greater biomass of litter inputs and the increased mulching affect associated with this treatment. Although not necessarily in labile forms, this treatment also received the highest input of organic N and P (Table 1) which may have led to increased growth. Alternatively, immobilization of N and P by the lower quality maize stover may have led to the observed increase in root biomass in the LQ treatment, with beneficial consequences for growth later in the season, when nutrients became more available.

Nitrogen uptake and movement in the soil

The significant influence of earthworms on the recovery of fertilizer N in surface soils and nearly all aggregate fractions (Figs. 5, 6 and 7) offers important insight as to how earthworms affect inorganic N sources. Several studies have shown earthworms to increase the availability of N following additions of inorganic N (Bohlen and Edwards 1995, Blair et al. 1997, Subler et al. 1998). Similar to our study, Bohlen et al. (1999) found *Lumbricus terrestris* to decrease the recovery of inorganic fertilizer in the surface layer (0-5cm) of microcosms (with no plants), suggesting that the added KNO_3 was mobilized by earthworm activity and transferred to lower soil layers. In the present study, earthworms decreased the proportion of fertilizer derived N, f, for nearly all aggregate fractions. Only in large macroaggregates was f not reduced by earthworm activity and was in fact increased by earthworms in the mM fraction (Fig. 5). As noted above, this fraction was largely responsible for the increases in total C and N observed for large macroaggregates in the presence of earthworms. We suspect that inorganic N entering the soil as fertilizer was partially immobilized by the microbial biomass associated with organic residues or by plant roots, both potential food sources for earthworms (Lee 1985). Thus, the higher level of ^{15}N in the mM fraction of large macroaggregates (Fig.6) further supports the idea that this fraction is a site of recently incorporated organic matter, particularly in the presence of earthworms. Alternatively, earthworms may directly consume mineral fertilizer, however, past findings suggest that earthworms do not consume or readily assimilate inorganic N in the absence of organic matter inputs (Bohlen et al. 1999, Fonte et al. 2007). Although, Fonte et al. (2007) found earthworms to decrease the incorporation of N derived from inorganic fertilizer into the mM fraction, mesocosms in their experiment were devoid of plants and organic inputs suggesting that earthworm food sources (organic resources) were largely unavailable to immobilize the added N and thus the added N was not incorporated into casts.

Although several studies have suggested that the increased mobility of N associated with earthworms can lead to leaching losses (Subler et al. 1997, Dominguez et al. 2004), there was no effect of earthworms on ^{15}N recovered below 15 cm in this study. Furthermore, there was no difference in total ^{15}N recovery (plants and soil) between any of the treatments in this experiment. The significant increase in plant uptake of applied inorganic N in the presence of earthworms, indicates that N availability was higher in the +W treatments and that plant uptake was responsible for the decrease in N recovered in the bulk soil (Fig. 7). Similar to our study, Baker et al. (2002) found earthworms to increase ^{15}N uptake, despite a lack of earthworm effect on plant biomass production. Thus it seems that earthworms help to maintain applied inorganic N in a more labile state, ultimately leading to increased uptake of this N source, regardless of effects on plant growth or total N uptake.

Plant litter quality did not affect the incorporation of applied inorganic N into the different fractions, but the presence or absence of litter did appear to play a minor role. Of greatest relevance, more ^{15}N was recovered in soils below 15 cm in the absence of plant litter, suggesting that organic residues may help to temporarily immobilize additions of inorganic N and ultimately decrease the loss of N from agroecosystems.

CONCLUSIONS

In the face of growing environmental degradation and increasingly limited resources, the need to identify more sustainable forms of agriculture is critical. Although long-term effects remain to be fully assessed, the Quesungual system appears to improve nutrient flow to the crop and to maintain SOM stocks via improved soil biological functioning. Based on the results of this study, we conclude that organic matter applications in the Quesungual system increase the benefit of the earthworm populations that they promote. Although we were not able to draw any firm conclusions about litter quality, the application of litter as mulch appears to interact positively with earthworms to improve soil structure and aggregate-associated SOM. The effects on nutrient cycling are less clear. The influence of earthworms on P dynamics in this study appears to conflict with past findings, thus emphasizing the need to evaluate the full effect of earthworms on this vital and often limiting nutrient in tropical systems. However, earthworms appear to play an important role in directing a greater proportion of applied inorganic nitrogen to the crop. Consequently, the integrated management of available plant residues and earthworms is needed to optimize SOM dynamics, nutrient cycling, and plant growth for sustainable agroecosystem functioning.

ACKNOWLEDGEMENTS

We would like to thank the Miguel Cruz for allowing us to conduct this research on his farm, as well as Edwin Garcia of the TSBF Institute of CIAT, Hector Vásquez and various field assistants that provided support while in Honduras. We also appreciate the support of everyone at CIAT in Tegucigalpa and Cali, who helped with planning and logistics, especially Dr. Edmundo Barrios who provided helpful advice in the early stages of this research. We also appreciate the assistance of many people in conducting laboratory work at UC Davis, including Kwun Luen Lai, Anglie Do, Katie Chun and Timothy Doane. This research was funded in part by a Fulbright student grant from the Institute of International Education. Partial support was also received from the Challenge Program on Water and Food of the CGIAR (PN-15). Additional research funds were provided by the UC Davis Henry A. Jastro and Peter J. Shields Graduate Research Scholarship and the National Science Foundation. Graduate student fellowships from the Kearney Foundation of Soil Science and the UC Davis, Department of Plant Sciences also provided support for a portion of this research.

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Table 1: Litter quality parameters and application rates of four litter treatments applied to soil mesocosms within the Quesungual agroforestry system of western Honduras. Treatments denoted by: Control (no plant litter added), Low Quality (maize stover), Mixed Quality (mixture of low and high quality litter), High Quality (leguminous *D. robinioides* litter).

	Litter Quality Parameters					**Amount Added**		
Litter Treatment	**C**	**N**	**P**	**Lignin**	**C:N**	**C**	**N**	**P**
	mg g^{-1}					**g m^{-2}**		
Control	-	-	-	-	-	0	0	0
Low Quality	431	17.6	3.2	47.7	24.5	176.7	7.20	1.30
Mixed Quality	437	21.0	2.8	52.9	20.8	114.8	5.5	0.74
High Quality	461	33.2	1.7	71.5	13.9	52.8	3.8	0.19

Table 2: Total phosphorus and indices of phosphorus availability for surface soils (0-15 cm) in mesocosms within the Quesungual agroforestry system in western Honduras. Treatments denoted by: Control (no litter applied and soil left bare), Litter Applied (the average of 3 litter quality treatments), +W (earthworms added), and –W (no earthworms). Numbers in italics below each average indicate standard error around the treatment mean.

Treatments		**P Measurements**		
Soil Cover	**Worm**	**Total P**	**Bray P**	**Olsen P**
		mg kg^{-1}		
Control	**+W**	200.0	5.1	4.6
		4.1	*0.3*	*0.3*
	-W	208.8	6.4	5.5
		3.1	*0.8*	*0.6*
Litter Applied	**+W**	199.0	4.6	3.9
		3.1	*0.3*	*0.3*
	-W	206.7	7.0	5.9
		2.5	*0.5*	*0.3*
ANOVA Table (P values[a])				
	Litter[b]	ns	ns	ns
	Worm	0.027	< 0.001	< 0.001
	Litter x Worm[b]	ns	0.007	0.005

[a] ns, P > 0.05

[b] P-values for both Litter and Litter x Worm effects are from orthogonal contrasts

Table 3: Biomass and nutrient uptake in maize plants grown in mesocosms within the Quesungual agroforestry system in western Honduras. Treatments denoted by: Control (no plant litter added), Low Quality (maize stover), Mixed Quality (mixture of low and high quality litter), High Quality (leguminous *D. robinioides* litter), +W (with earthworms), and –W (no earthworms). Numbers in italics below each average indicate standard error around the treatment mean.

Treatments		**Biomass of Plant Components**				**Whole Plant Nutrient Uptake**		
Litter	**Worm**	**Total**	**Grain**	**Non-grain**	**Root**	**N**	**P**	**Fert N[a]**
		g				mg		
Control	**+W**	173.8	43.4	117.6	12.8	1881	227	569
		13.2	*20.5*	*15.3*	*4.3*	*168*	*28*	*19*
	-W	176.3	53	110.4	12.8	1870	222	543
		3.5	*20.4*	*20.2*	*2.7*	*50*	*20*	*11*
Low Quality	**+W**	270.4	42.6	204.9	22.8	3171	358	631
		21.5	*12.6*	*28.0*	*3.1*	*453*	*64*	*11*
	-W	216.1	53.6	145.8	16.7	2280	252	564
		11.7	*12.1*	*11.1*	*2.0*	*279*	*30*	*22*
Mixed Quality	**+W**	175.5	53.7	109.7	12.0	1899	238	588
		32.0	*38.4*	*27.9*	*7.6*	*264*	*18*	*23*
	-W	198	61.3	122.3	14.4	2119	254	557
		15.5	*30.8*	*34.6*	*2.3*	*156*	*20*	*24*
High Quality	**+W**	176.3	45.1	118.5	12.7	2047	248	576
		17.5	*11.8*	*25.3*	*1.9*	*310*	*29*	*29*
	-W	162.5	55.5	96.3	10.7	1832	222	552
		16.7	*26.7*	*39.0*	*2.9*	*178*	*14*	*9*
ANOVA Table (P values[b])								
	Litter	0.003	ns	0.001	0.002	0.008	ns	ns
	Worm	ns	ns	ns	ns	ns	ns	0.018
	Litter x Worm	ns	ns	ns	ns	ns	ns	ns

[a] Total N in maize plant derived from ^{15}N labeled inorganic fertilizer application

[b] ns, $P > 0.05$

FIGURE LEGENDS

Figure 1: Side view of experimental mesocosm used to manipulate earthworm and litter treatments within the Quesungual agroforestry system in western Honduras, June to September 2007.

Figure 2: Aggregate stability for surface soils (0-15 cm) from various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (control= no plant litter added), LQ (low quality maize stover), MQ (mixed low and high quality litter), HQ (high quality litter), + (with earthworm), and – (no earthworms). Error bars represent the standard error of each treatment mean.

Figure 3: Carbon content of aggregate fractions in surface soils (0-15 cm) sampled from various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (Control, no litter applied and soil left bare), LA (Litter Applied, the average of 3 litter quality treatments), + (earthworms added), and – (no earthworms). Error bars represent the standard error around each treatment mean.

Figure 4: Carbon content of large macroaggregate occluded fractions of surface soils (0-15 cm), sampled from various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (Control, no litter applied and soil left bare), LA (Litter Applied, the average of 3 litter quality treatments), + (earthworms added), and – (no earthworms). Error bars represent the standard error around each treatment mean.

Figure 5: Proportion of N in aggregate fractions of surface soils (0-15 cm) derived from inorganic ^{15}N additions, for various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (Control, no litter applied and soil left bare), LA (Litter Applied, the average of 3 litter quality treatments), + (earthworms added), and – (no earthworms). Error bars represent the standard error around each treatment mean.

Figure 6: Proportion of N in large macroaggregate occluded fractions of surface soils (0-15 cm) derived from inorganic ^{15}N additions, for various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (Control, no litter applied and soil left bare), LA (Litter Applied, the average of 3 litter quality treatments), + (earthworms added), and – (no earthworms). Error bars represent the standard error around each treatment mean.

Figure 7: Recovery of ^{15}N labeled fertilizer in soils and maize plants from various litter and earthworm treatment combinations within the Quesungual agroforestry system in western Honduras. Treatments denoted by: C (Control, no litter applied and soil left bare), LA (Litter Applied, the average of 3 litter quality treatments), + (earthworms added), and – (no earthworms). Error bars represent the standard error around each treatment mean.

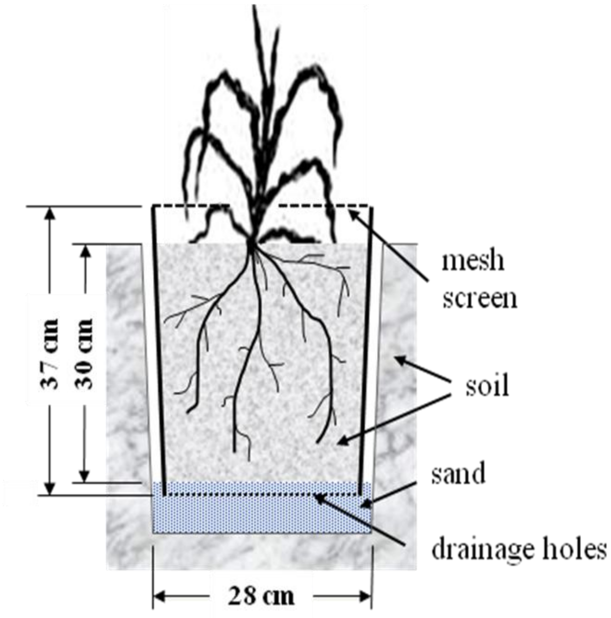


Figure 1

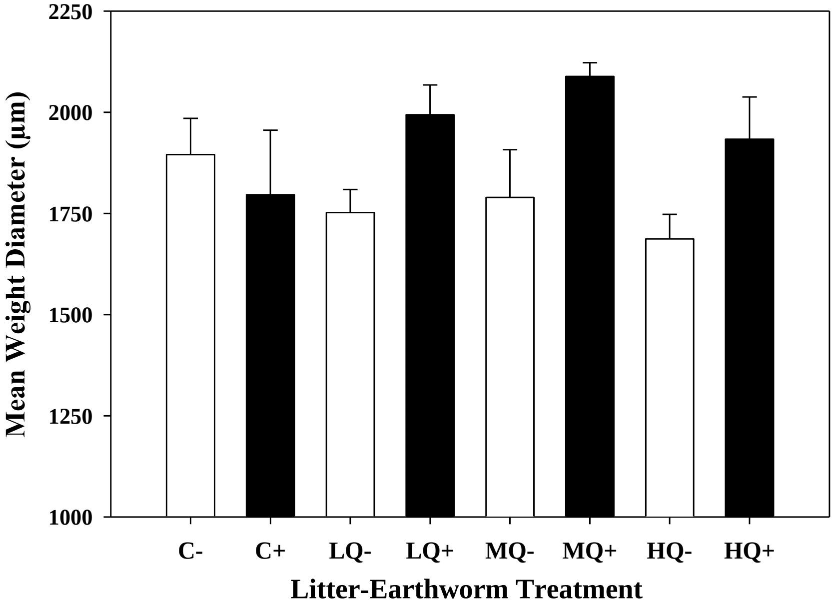

Mean Weight Diameter (µm)
2250
2000
1750
1500
1250
1000
C-
C+
LQ-
LQ+
MQ-
MQ+
HQ-
HQ+
Litter-Earthworm Treatment

Figure 2

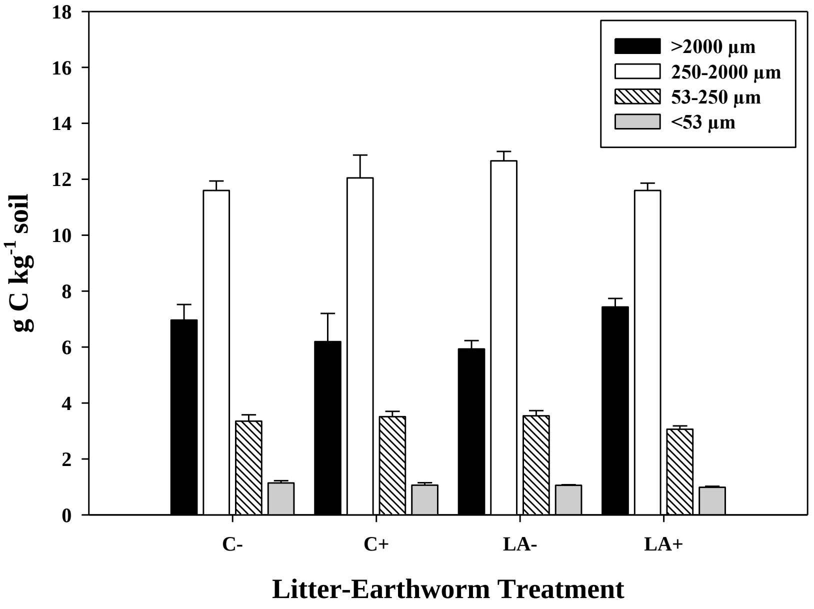
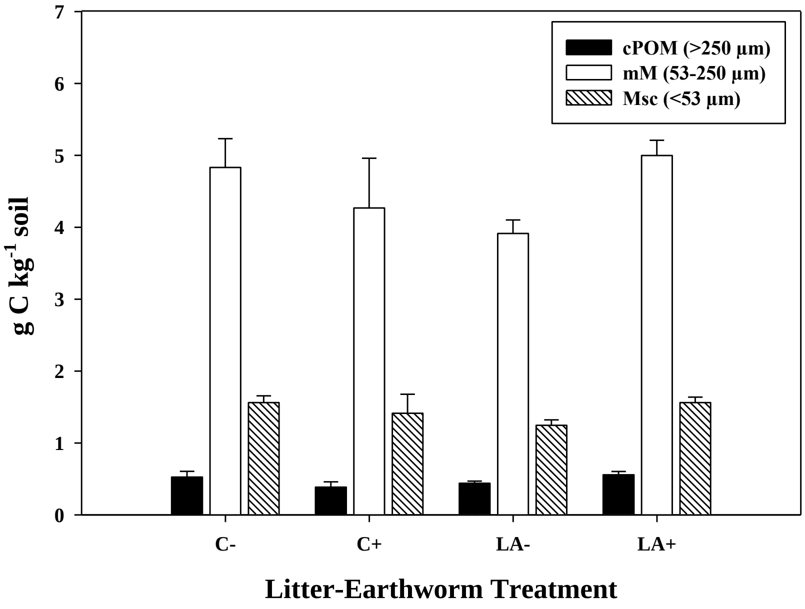

18
16
14
12
10
8
6
4
2
0
g C kg^{-1} soil
>2000 µm
250-2000 µm
53-250 µm
<53 µm
C-
C+
LA-
LA+
Litter-Earthworm Treatment

Figure 3



7
6
5
4
3
2
1
0
g C kg^{-1} soil
cPOM (>250 µm)
mM (53-250 µm)
Msc (<53 µm)
C-
C+
LA-
LA+
Litter-Earthworm Treatment

Figure 4

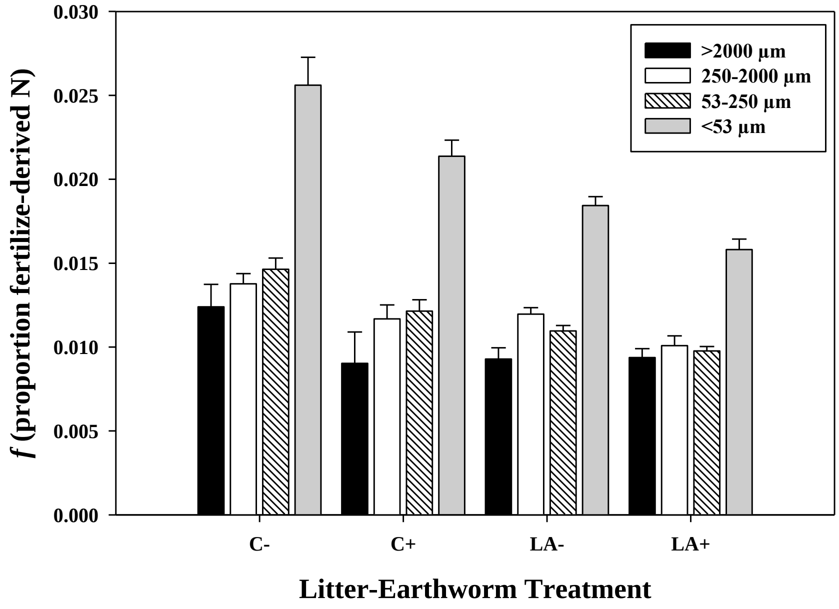

0.030
0.025
0.020
0.015
0.010
0.005
0.000
f (proportion fertilize-derived N)
>2000 µm
250-2000 µm
53-250 µm
<53 µm
C-
C+
LA-
LA+
Litter-Earthworm Treatment

Figure 5

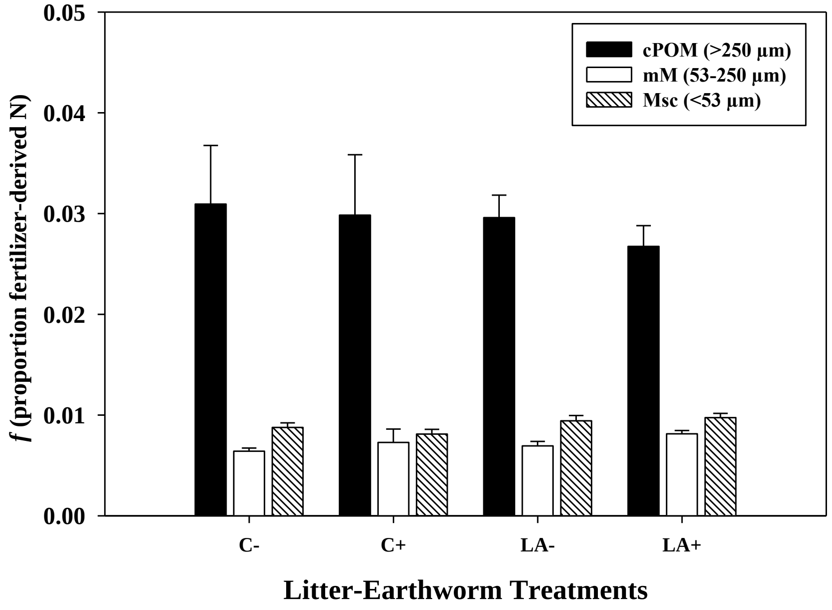

0.05
0.04
0.03
0.02
0.01
0.00
f (proportion fertilizer-derived N)
■ cPOM (>250 µm)
☐ mM (53-250 µm)
▨ Msc (<53 µm)
C-
C+
LA-
LA+
Litter-Earthworm Treatments

Figure 6

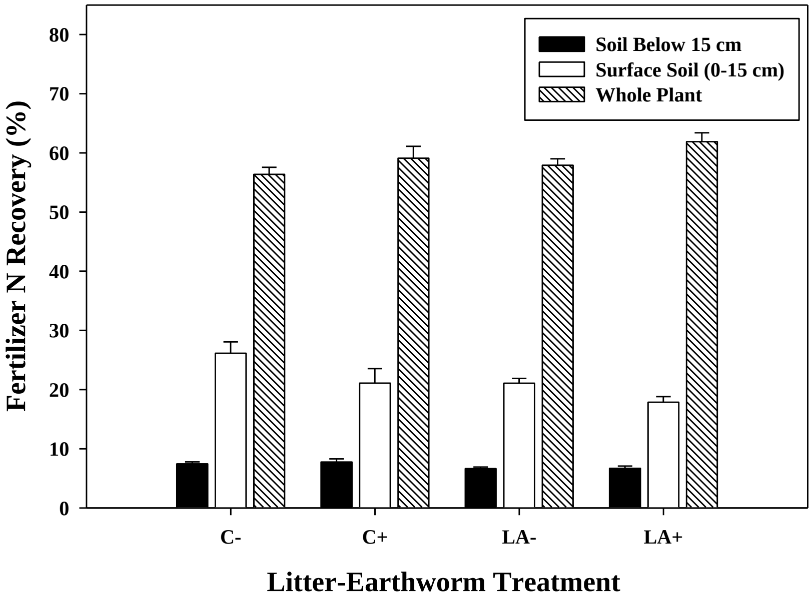

Fertilizer N Recovery (%)
0
10
20
30
40
50
60
70
80
Soil Below 15 cm
Surface Soil (0-15 cm)
Whole Plant
C-
C+
LA-
LA+
Litter-Earthworm Treatment

Figure 7