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Endogeic earthworm densities increase in response to higher fine-root production in a forest exposed to elevated CO_2

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ABSTRACT

Net primary productivity (NPP) influences soil food webs and ultimately the amount of carbon (C) inputs in ecosystems. Earthworms can physically protect organic matter from rapid mineralization through the formation of soil aggregates. Previous studies at the Oak Ridge National Laboratory (ORNL) Free Air CO_2 Enrichment (FACE) experiment showed that elevated [CO_2] (e[CO_2]) increased fine-root production and increased soil C through soil aggregation compared to ambient [CO_2] (a[CO_2]) conditions. Our first objective was to study the response of earthworms to increased leaf and root-litter inputs caused by increased atmospheric [CO_2] exposure. We also took advantage of the CO_2 shutdown at the ORNL FACE site to track the shift of the $\delta^{13}C$ signal in leaf-litter, fine roots, earthworms, earthworm casts, and bulk soil. Densities of the most abundant endogeic earthworm, *Diplocardia* spp., were positively correlated with the previous-year production of leaf litter ($r = 0.66$, $P = 0.02$) and fine roots ($r = 0.62$, $P = 0.03$); and with the leaf-litter production ($r = 0.63$, $P = 0.03$) and fine-root production ($r = 0.59$, $P = 0.05$) two years before earthworms were sampled. Within two years after the CO_2 fumigation ceased, the $^{13}C/^{12}C$ ratio increased in leaf litter ($P = 0.01$) and in fine roots ($P = 0.05$), showing an ecosystem legacy effect on soil C inputs. However, the C isotopic composition of soil, endogeic earthworms and casts had not changed the two years after the CO_2 fumigation ended. The positive response of earthworms to increased root NPP, caused by elevated [CO_2], is consistent with the increased soil aggregate formation and increased soil C at the ORNL FACE in the e[CO_2] treatment.

1. Introduction

One of the most pronounced global effects of human activity is the sharp increase in atmospheric CO_2 concentration (Schulze, 2006). Elevated atmospheric CO_2 concentration has a direct impact on global vegetation by increasing productivity, with potentially important cascading effects on soil organisms (Gonzalez-Meler et al., 2004; Norby and Zak, 2011). Many studies have evaluated plant responses to elevated [CO_2] (e.g. Norby and Zak, 2011; Iversen et al., 2012), but very few have examined effects on the belowground macrobiota. One of the biggest uncertainties is how the soil macrofauna respond to ecosystems exposed to elevated [CO_2], despite the recognized role of the macrofauna in processing plant litter, and incorporating and stabilizing organic matter in soils. Knowing the feedbacks and interactions among biotic and edaphic processes that determine the strength of an ecosystem to capture and maintain carbon (C) becomes critical for increasing the predictive capability of models of global change. The soil macrofauna may be a substantial player in these feedbacks.

Research at the Oak Ridge National Laboratory (ORNL) Free-Air CO_2 Enrichment (FACE) site has shown that forest net primary productivity (NPP), particularly the fraction of NPP attributable to fine-root production, increases in response to elevated atmospheric CO_2 concentrations (e[CO_2]) (Matamala et al., 2003; Norby et al., 2004; Iversen et al., 2008). However, the CO_2-induced enhancement of NPP was not sustained as nitrogen availability in the forest declined (Norby et al., 2010a). Changes in NPP can directly affect the quantity and

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quality of organic inputs to the soil, potentially leading to cascading effects on the soil food web and ultimately on C cycling. Despite the known influence of the soil macrofauna on soil processes, the effects of elevated atmospheric [CO_2] on the macrofauna and feedbacks between the soil macrofauna and biogeochemical cycles in forest ecosystems are largely unknown (Scullion et al., 2014).

Because activities of the soil macrofauna can influence biogeochemical processes (Lavelle et al., 2006; Coleman, 2008), neglecting responses of the soil macrofauna to changes in atmospheric [CO_2] overlooks a potentially critical component of the ecosystem response. Earthworms, which are major constituents of the soil macrofauna, can impact soil processes disproportionately to their density or biomass (Lavelle et al., 2006; Brussaard et al., 2007). Through their feeding, casting and burrowing behavior, earthworms can influence decomposition, C and nutrient cycling, and the maintenance of soil structure (Blair et al., 1995; Groffman and Bohlen, 1999; Lavelle et al., 2006). Earthworm species have been placed in one of three major ecophysiological groups (epigeic, anecic and endogeic) based primarily on their soil microhabitat and burrowing and feeding behaviors (Bouché, 1977; Blair et al., 1995; Lavelle, 2002; Coleman et al., 2004). Epigeic earthworms are litter feeders that live in the litter layer. Anecic earthworms, which are litter feeders that live primarily in mineral soil, form deep vertical burrows and feed mainly on surface litter that they transport deeper into the soil profile. Endogeic earthworms are soil feeders that live in the mineral soil. Intermediate categories exist for species that do not completely fit within one of the major ecological categories; for example, epi-endogeic earthworms inhabit the surface soil and consume plant litter (Coleman et al., 2004).

Earthworms can increase soil-C sequestration by producing casts (Lee, 1985; Edwards and Bohlen, 1996; Frelich et al., 2006), which become soil aggregates that protect organic C from microbial decomposition (Bossuyt et al., 2005; Sánchez-de León et al., 2014). The ORNL experiment was one of only a few FACE studies to have documented increased soil-surface C under elevated [CO_2] (Jastrow et al., 2005; Iversen et al., 2012), including an increase in microaggregated C (Jastrow et al., 2005). Thus, the ORNL FACE site offered an almost unique opportunity to investigate whether increased earthworm densities could help explain the increased C seen in surface soils under elevated [CO_2].

Our first objective was to study how changes in the production of plant litter affects earthworms. We hypothesized that because of increased belowground production resulting from higher rates of organic-matter input under elevated [CO_2] (e[CO_2]) conditions, endogeic earthworms would be more abundant in e[CO_2] than ambient [CO_2] (a[CO_2]) treatments. We also hypothesized that because of time lags in the endogeic earthworm response, there would also be a correlation between previous rates of plant litter production and current earthworm abundance. Our second objective was to establish a connection between increased belowground production and earthworm densities by tracking C from plant litter to earthworm tissues and casts using the stable isotope ^{13}C tracer (Pataki et al., 2003). We were able to accomplish this because the e[CO_2] plots had a unique $\delta^{13}C$ signature after 12 years exposure to a ^{13}C-depleted CO_2 source. In addition, Lynch et al. (2013) found that new fine roots started to show "relaxation" (i.e. loss) of the $\delta^{13}C$ signal as early as 6 months after the cessation of the CO_2 fumigation at the Oak Ridge FACE site. We tracked the transfer of the ^{13}C allocated to leaf litter and fine roots to earthworms and then to soil organic matter (SOM) via earthworm casts. We hypothesized that earthworm isotopic composition would shift in a manner reflecting the turnover rate of its source of C. Thus, the $\delta^{13}C$ signal of epigeic, anecic and epi-endogeic earthworms – all of which consume plant litter – would shift after one year of the cessation of CO_2 fumigation. For endogeic earthworms, which feed upon SOM, we hypothesized that the $\delta^{13}C$ signal would shift after incorporation of plant litter into SOM, which would require at least 2 years.

2. Materials and methods

2.1. Study site and experimental design

The ORNL FACE site was located in a sweetgum (*Liquidambar styraciflua* L.) plantation that had been established in 1988 in the Oak Ridge National Environmental Research Park in Roane County, Tennessee, USA (35°54′ N; 84°20′ W). The soils are classified as Aquic Hapludult with silty clay loam texture (Soil Survey Staff, 2018). The FACE site consisted of two 25 m diameter plots that experienced air with e[CO_2] from April 1998 until September 2009 (12 growing seasons), and two 25 m diameter control plots with similar infrastructure and ambient CO_2 concentrations (a[CO_2]) (Norby et al., 2006; Riggs et al., 2009). An additional control plot without the FACE infrastructure was not used in this analysis. The CO_2 enrichment was achieved using the Brookhaven National Laboratory design (Hendrey et al., 1999) as described by Norby et al. (2001). Pure CO_2 was released through vertical vent pipes surrounding the plots, with the rate of release dependent on wind speed and a feedback control. The atmospheric CO_2 concentration of the e[CO_2] treatment averaged 547 ppm during the experiment, and [CO_2] in the ambient plots averaged 395 ppm (Riggs et al., 2009; Walker et al., 2014). The elevated [CO_2] treatment was continuous during daylight hours during each growing season (April through November) until September 2009.

The CO_2 used in the e[CO_2] treatment was $\delta^{13}C$-depleted with an $\delta^{13}C$ signature of approximately −50‰ (Norby et al., 2006) which is incorporated into tissues and fluxes during the experiment (Matamala et al., 2003; Lynch et al., 2013; Gonzalez-Meler et al., 2014). Other ecological data, such as root productivity, litter fall, root mortality and tissue nitrogen concentration were periodically collected (Ledford et al., 2008; Norby et al., 2010b).

2.2. Estimating earthworm densities

Earthworm sampling was conducted in September 2007, May 2008, July 2008, October 2008, May 2010, October 2010 and May 2011, when moist and warm soil conditions promote earthworm activity. We used a combination of hand sorting and extraction with a solution of allyl isothiocyanate (AITC) in four sub-samples within each of the four plots (i.e. two e[CO_2] plots and two a[CO_2] plots). The AITC was selected over formalin (most commonly used extractant) because of AITC's documented efficacy (Zaborski, 2003) and the fact that it is not toxic to earthworms, researchers or the soil environment (Valckx et al., 2011). We first irrigated the soil with a solution of AITC (100 mg L^{-1}), isopropanol and water (Zaborski, 2003) over an area of 0.06 m^2 (25 cm × 25 cm) to drive worms to the surface. After 10 min, we manually excavated the 25 cm × 25 cm area to a depth of 10 cm, placed the soil over a plastic sheet and sorted earthworms by hand. We then did a second application of the AITC solution in the soil pit to bring to the surface earthworms that were below the 10 cm depth. Earthworms were taken to the laboratory, classified to morphospecies, counted, and their fresh weight was measured. Selected adult specimens were preserved in a formalin solution (formaldehyde 1:10 solution) (James, 1999) for taxonomic identification. Thus, earthworm density was expressed both in terms of numbers and a measure of biomass (fresh weight).

2.3. Preparation of earthworm tissue and casts for isotope analysis

Methods for preparation of earthworm tissues and casts for isotope analysis were adapted from Schmidt (1999). Earthworms that were not used for taxonomic identification were left in Petri dishes with glass-fiber filters (Fisherbrand® Glass Fiber Filter Circles G6), moistened with a diluted 1:8 solution of frog Ringer's Solution for 3 days to allow them to empty their guts (Schmidt, 1999). The filter paper was removed daily and dried at 60 °C for 15 min or until the casts were dry enough to be