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Original article

Long-term consequences of biochemical and biogeochemical changes in the Horseshoe Bend agroecosystem, Athens, GA

D. Coleman*, M. Hunter, P. Hendrix, D. Crossley Jr., B. Simmons, K. Wickings

Institute of Ecology, University of Georgia, Athens, GA 30602, USA

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Abstract

Understanding many soil processes, including the accumulation of organic matter and the formation and loss of soil aggregates, requires research that is conducted over decadal time periods. The dynamics of soil organic matter and soil fauna at the Horseshoe Bend (HSB) agroecosystem site in Georgia have been studied in replicated experimental plots since 1978. The experimental treatments (no-tillage (NT) and conventional-tillage (CT) regimes) are continuing to diverge in amounts and distribution of SOM in the soil profiles of HSB. Our current research focuses on two major areas: 1) long-term measurements of the gradually-increasing base of soil organic matter from C3-pathway plants, in crop rotations that have been in effect since 1997; 2) following the production, accumulation, fate and ecological effects of the *Bt* (*Bacillus thuringiensis*) proteins from the summer planting of *Bt* (and non-*Bt*) cotton in subplots within our main plots. The variation in the size of soil aggregates may influence the sequestration of *Bt* toxins, and their breakdown products, within soils. NT management systems at HSB generate an increasing proportion of soil macroaggregates in comparison to the markedly reduced macroaggregates in our CT plots. We suggest that NT systems may sequester more *Bt*-related products than will CT plots.

Keywords: *Bt*; CryIA(c); Macroaggregates; Microaggregates; Soil carbon sequestration; Soil biota

1. Introduction

1.1. Biogeochemical studies: long-term changes in soil organic carbon

Several soil scientists have used the natural signal from C3-type vegetation (δ^{13}C ca. –26‰) in the indigenous soil organic matter, and then followed the change that occurs from growing C4-type vegetation (δ^{13}C ca. –12‰) in experimental fields [1,2]. Our studies have used a modified approach, converting from a regime of C3 winter cover crops (wheat, rye, clover) and C4 summer maize or grain sorghum crops, to all C3 winter crops and summer (kenaf, cotton) crops. We are following changes in isotopic ratios of some soil C pools, measuring the long-term accretion of C in various soil fractions [18,23,30] in our agroecosystem. This research on a sub-tropical soil serves as a useful comparison and contrast to the results of Balesdent et al. [1,4] in temperate soils and Cerri et al. [11] in tropical soils. We expect that C stabilization (i.e. slower C turnover) will be more pronounced over the long-term in NT than in CT plots due to C protection within microaggregate fractions in the upper soil stratum of NT. We are particularly interested in the influence of soil fauna on this process, and its impacts on flows of

* Corresponding author. Tel.: +1 706 542 2309;
fax: +1 706 583 0646.
E-mail address: David.Coleman@csiro.au (D. Coleman).

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carbon and nitrogen in soil food webs [9,12]. Possible interactions between Bt CryIA(c) proteins and breakdown products and their possible effects on key indicator soil mesofauna [12] are also of concern.

1.2. Long-term application of Bt crop residues

Although there has been some research on the effects of transgenic plants on soil communities and ecosystem function, studies on belowground effects of transgenic plants lag far behind those above ground [19]. Both crop residues and root exudates can transfer the products of transgenes below ground. For example, transformed (NK4640*Bt*) corn plants exude *Bt* from their roots both in vitro and in situ [26–28]. The exudates are lethal to *Manduca sexta* larvae used as model Lepidopterans and particles of rhizosphere soil from experimental plants are also lethal to larvae. The experiments demonstrate that *Bt* exuded from roots can remain biologically active. *Bt* can remain active in soils because it binds rapidly and tightly to clays and humic acids [15,33], where it is protected from microbial degradation [20] and may even be taken up again by plants [25,28]. Activity in soil can remain for at least 234 days [32], the longest time for which tests have been reported to date. We believe that this is not long enough. What are the long-term (multiple-year) effects of *Bt* breakdown products in soil? Perhaps *Bt* in soils will benefit crop production by acting against root-feeding pests or soil-dwelling stages of aboveground pests. But the potential for deleterious effects upon beneficial soil fauna and flora remains to be examined rigorously over extended periods of exposure. Donegan et al. [16] reported changes to soil ecosystems in response to 2 years of exposure to transgenic alfalfa. They compared the effects of untransformed, transgenic α-amylase-producing, and transgenic lignin peroxidase-producing alfalfa on soil chemistry, flora, and fauna. Under alfalfa transformed to express lignin peroxidase, they detected higher population levels of culturable aerobic spore-forming and cellulose-utilizing bacteria, lower activity of the soil enzymes, dehydrogenase and alkaline phosphatase, and higher soil pH levels. Changes in the populations of rhizosphere bacteria in response to transgenic crops have been reported a number of times [22,24]. However, Donegan et al. [16] reported no effects of transgenic alfalfa on soil protozoa, nematodes, microarthropods, or soil respiration. Their results support previous work in which laboratory populations of two species of Collembola were unaffected by exposure to four different *Bt* toxins [29]. Indeed Saxena and Stotzky [25] concluded that *Bt* toxin released from corn had no influence on earthworms, nematodes, protozoa, bacteria or fungi in soil. Longer-term impacts are yet to be measured, and we are following short-term and long-term aboveground and belowground responses, as presented below.

2. Materials and methods

2.1. The Horseshoe Bend (HSB) field site

HSB is a two ha. research site of the University of Georgia, situated in bottomland (sandy loam, typic Kanhapludult) along the middle fork of the Oconee River, in Athens-Clarke County, GA. Mean annual precipitation is 1400 mm, and annual mean minimum and maximum temperatures are 8.3 and 19.3 °C for conventional-tillage (CT) and 9.5 and 17.5 °C for no-tillage (NT) plots [12]. Research has been conducted continuously at HSB since Odum et al. [21] set up old fields in the mid-1960s. From 1978 onward, four 0.1 ha plots have been managed with moldboard plowing (to 15 cm.) followed by disking (CT), and another four 0.1 ha. plots have been managed using a NT regime, with the only soil disturbance being direct seed drilling in these untilled plots. We sow winter cover crops of wheat and crimson clover, and various summer crops, including corn (*Zea mays* L.), and beginning in 1999, cotton (*Gossypium hirsutum* L.), either engineered *Bt* (producing the Cry1A(c) protein), or non-*Bt*. The NT plots have built up a significant organic layer near the soil surface, and tend to be dominated by fungal tissues in the top 1–2 cm [5,7]. In contrast, the CT plots have a more uniform distribution of the organic carbon in the soil profile in the top 15-cm (Table 1). The tillage and Bt treatments are set up in a split–split plot design.

2.2. Sampling and analytical procedures

For soil organic matter analyses, samples were taken from CT and NT plots in quadruplicate for each 0.1 ha plot, for a total of 16 samples at 0–2.5, 2.5–5 and 5–15 cm depths. Samples were kept at 4 °C for no more

Table 1
Some general properties of CT and NT soils at the HSB Experimental Area, Athens, GA, USA

Depth (cm)	Organic C ($g\ kg^{-1}$)		Organic N ($g\ kg^{-1}$)		Bulk density ($mg\ m^{-3}$)	
	NT	CT	NT	CT	NT	CT
0–5	24.2	10.9	2.23	1.08	1.20	1.38
5–15	10.1	8.2	0.84	0.83	1.52	1.51