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Aggregate-associated soil organic matter as an ecosystem property and a measurement tool*



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ABSTRACT

Our 2000 paper Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture had its genesis in attempts to identify and isolate soil organic matter (SOM) fractions that reflect the impacts of climate, soil physiochemical properties and physical disturbance on the soil organic carbon balance. A key prerequisite for the investigation was the development of a simple device to isolate the microaggregates (53-250 μm) contained within stable (i.e., resistant to slaking) macroaggregates (>250 µm) obtained by conventional wet-sieving. By comparing the abundance and C content of micro-within-macroaggregates, the size distribution of intra-aggregate particulate organic matter (iPOM) and isotopically-based estimates of the age of the organic matter in the different fractions, we were able to corroborate our hypothesis that the absence of tillage (i.e., in no-till and native soils) promotes greater longevity of newly-formed macroaggregates, resulting in greater SOM stabilization in microaggregates formed within stable macroaggregates. Follow-up research has indicated that the microaggregate-within-macroaggregate fraction is 1) potentially a robust indicator for management-induced SOC changes over decadal time scales, 2) of biological origin and therefore useful in interpreting impacts of soil biota on soil C and N dynamics, but not in-situ CO2 and N2O fluxes, 3) useful in complimentary chemical and spectroscopic approaches to relate SOM dynamics to soil structure and attributes of the soil pore space, and 4) a good candidate for being incorporated into models as a measurable fraction.

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1. Introduction

During the 1980s and 90s, two topics of debate came into the mainstream of soil organic matter SOM research, both of which continue to be widely discussed and studied to the present day. One was the issue of how to characterize SOM in terms of its components in ways that better reflected ecosystem-level perturbations such as tillage, land cover change and climate change. There was a growing consensus that the fulvic and humic acids revealed by classical chemical extraction processes were not representative of 'functional' SOM fractions (Elliott et al., 1996). This realization sparked an interest in physically-defined SOM fractions, whereby the actual location of organic matter within the soil matrix and the size and density of different organo-mineral complexes were

viewed as key attributes determining the function and turnover of SOM. The second topic arose from the recognition that soil C sequestration might be a viable option for greenhouse gas and climate change mitigation (Paustian et al., 1997). Hence, there was a greater imperative to elucidate the underlying mechanisms for soil C sequestration under conservation management practices and develop means to better measure and predict soil C stocks changes as a function of management and environmental drivers. In the US, there was particular interest in the effects of no-tillage management and land use change (e.g. from native to cropped) on SOM-C stocks (Bruce et al. 1999; Lal et al. 1999).

It is within this framework that the research described in our paper (Six et al., 2000a) was developed, conducted and interpreted. We sought to elucidate how changes in aggregation were linked to soil C sequestration under no-tillage compared to conventional tillage management. This link between aggregate structure and SOM dynamics had been investigated before (Elliott, 1986), but here we developed a new method to isolate a specific fraction, i.e., the microaggregate-within-macroaggregate. Six et al. (1998, 1999) hypothesized that this aggregate fraction is a fraction in which

 $[\]mbox{\ensuremath{\,^{\dot{\ensuremath{\,^{\circ}}}}}}\xspace$ Dedicated to Dr. Ted Elliott who was instrumental in developing our Citation Classic.

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soil C is preferentially stabilized in no-tillage soils. The hypothesis was tested and corroborated across four different sites, allowing us to generalize, the proposed mechanism for soil C sequestration with no-tillage management.

2. Background

The theoretical and methodological framework for our paper stems most directly from the Elliott (1986) paper on aggregate structure and organic matter in native and cultivated soils in the US Great Plains. Elliott had long been interested in the physical structure of the soil as it relates to SOM and the dynamics of the soil decomposer community, as evidenced by his seminal paper on habitable pore space and trophic interactions in soil (Elliott et al., 1980). Later, while working on the National Science Foundation "Great Plains Project" (one of the first regional-scale agroecosystem studies), Elliott was inspired by the conceptual model of soil aggregate hierarchy presented by Tisdall and Oades (1982) and its potential applicability to soils in the Great Plains, where the generally negative effects of land use change from prairie to annual cropland were a main focus. He found patterns consistent with the Tisdall and Oades (1982) model, including higher C contents in macroaggregates compared to microaggregates, but that the 'additional' C was subject to rapid mineralization following aggregate disruption. Subsequent papers together with Cambardella (Elliott and Cambardella, 1991; Cambardella and Elliott, 1992, 1993a,b, 1994) defined and investigated physically-based fractions (using size and density fractionation), including particulate organic matter (POM) and a mineral-associated fraction, termed the enriched labile fraction (ELF). These fractions were postulated to represent SOM pools with 'intermediate' turnover times (i.e., several years to a few decades) and, in fact, the bulk of the SOM impacted by land use and management.

It was September 1995, when Six joined Elliott and Paustian at the Natural Resource Ecology Laboratory (NREL) in Fort Collins, Colorado to start his PhD research within a US National Science Foundation project titled "Environmental and Management Controls on Soil Structure and Organic Matter Dynamics". The global objective of the project was to understand the controls on these 'intermediate' SOM fractions and to quantify their role in organic matter and nutrient dynamics in agroecosystems — particularly with respect to soil disturbance (e.g., tillage) and its impact on soil structure and SOM dynamics. It must have been in one of the first weekly meetings between student and advisors that Elliott told Six: "In the beginning of your PhD work on this project, I will teach you a lot, but your goal is to teach me something new by the end of your PhD!". This statement was a continuous driving force throughout Six's PhD.

In the first few experiments (Six et al., 1998, 1999, 2000c), soils from four different sites with a no-tillage, conventional tillage, and native vegetation treatment were fractionated into different aggregate size classes and aggregate-associated SOM fractions (POM and ELF) with a methodology similar to that used by Cambardella and Elliott (1993a,b, 1994) and Jastrow (1996). The main modification in the methodology was that intra-aggregate POM (iPOM) was split in two different fractions: coarse iPOM and fine iPOM. This division was the result of wanting to have two different size classes of sand (with which the two different size classes of iPOM are associated) to develop what was called the Normalized Stability Index (Six et al., 2000b). But once this separation was done, it was thought that the POM-C associated with the coarse and fine sand fractions could as well be measured instead of having, as originally intended, one combined fraction of iPOM. The expedient decision to separately analyze the two iPOM fractions turned out to be a lucky move because it formed the basis for using

the fine to coarse iPOM ratio as an indicator for macroaggregate turnover and its influence on soil C stabilization. It was the higher ratio of fine to coarse iPOM in no-tillage compared to conventional tillage soils that suggested a slower macroaggregate turnover in notillage, leading to greater stabilization of C in microaggregateswithin-macroaggregates (Six et al., 1999). However, to really test the preferential stabilization of C in microaggregates-withinmacroaggregates when macroaggregate turnover is slower due to less soil disturbance, a method was needed to isolate the microaggregates occluded within macroaggregates. One morning, some discarded materials (e.g. mesh, a funnel, plexiglass tubing, regular tubing, etc.) lying around in the Natural Resource Ecology Laboratory were used to construct the "microaggregate isolator" (Six et al., 2000a) and a few days later microaggregates contained within macroaggregates were isolated from all four soils. Once their C was measured, the preferential stabilization of C in microaggregates in no-tillage soils was corroborated and formed the foundation for what was to become the Soil Biology & Biochemistry Citation Classic paper. Upon seeing the results Elliott acknowledged that he had unsuccessfully tried for many years to isolate microaggregateswithin-macroaggregates, but now that it was finally accomplished it corroborated some long-standing hypotheses. He said: "You've done what I have been trying to do for the last 15 years!". And there was a sense of the student having taught something to the advisor...

3. Opportunities and pitfalls

In the years following the publication of our *Soil Biology & Biochemistry* Citation Classic paper, several advances have been made. As a result, it has become clear what the strengths and weaknesses were of both the original methodology and its implications for mechanistic understanding of soil C stabilization.

3.1. A diagnostic fraction for management effects on carbon storage

As indicated above, the results from four temperate soils under both long-term no-tillage and conventional tillage management indicated that greater amounts of C were protected inside the microaggregate-within-macroaggregate fraction in no-tillage soils. In follow-up work involving three no-tillage and conventional tillage experiments in widely varying soil types (Mollisol, Alfisol, Oxisol) and environments (i.e. Nebraska, Kentucky, Brazil), we obtained further support for this preferential C stabilization by finding that over 90% of the difference in total SOC between no-tillage and conventional tillage could be accounted for by C associated with the microaggregates-within-macroaggregates (Denef et al., 2004) (Table 1). In a fourth experiment (also in Brazil) on a highly weathered Oxisol, we found that after five years of no-tillage there was no significant increase in microaggregate-within-macroaggregate C relative to conventional tillage, nor was there any difference in total SOC levels between no-tillage and conventional tillage (Denef et al., 2007). These initial findings suggested that this microaggregatewithin-macroaggregate C fraction may be a diagnostic fraction for changes in total SOC in response to changes in tillage management practices. In more recent studies (Table 1), we have further corroborated that microaggregate-within-macroaggregate C is a general diagnostic for management-induced changes in SOC levels in agroecosystems on a wide range of soil types and under drastically different climates (See Table 1). Hence, it represents an indicator of SOC storage capacity of best management practices as affected by factors, such as climate, soil type and management history. Furthermore, this fraction could provide a focal point for further elucidating mechanisms of SOM stabilization and turnover in soil, help explain the variability in the capacity of different soils to

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