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Iron-mediated stabilization of soil carbon amplifies the benefits of ecological restoration in degraded lands

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Abstract. Recent observations across a 14-year restoration chronosequence have shown an unexpected accumulation of soil organic carbon in strip-mined areas of central Brazil. This was attributed to the rapid plant colonization that followed the incorporation of biosolids into exposed regoliths, but the specific mechanisms involved in the stabilization of carbon inputs from the vegetation remained unclear. Using isotopic and elemental analyses, we tested the hypothesis that plant-derived carbon accumulation was triggered by the formation of iron-coordinated complexes, stabilized into physically protected (occluded) soil fractions. Confirming this hypothesis, we identified a fast formation of microaggregates shortly after the application of iron-rich biosolids, which was characterized by a strong association between pyrophosphate-extractable iron and plant-derived organic matter. The formation of microaggregates preceded the development of macroaggregates, which drastically increased soil carbon content (~140 Mg C/ha) a few years after restoration. Consistent with previous theoretical work, iron-coordinated organic complexes served as nuclei for aggregate formation, reflecting the synergistic effect of biological, chemical, and physical mechanisms of carbon stabilization in developing soils. Nevertheless, iron was not the only factor affecting soil carbon content. The highest carbon accumulation was observed during the period of highest plant diversity (>30 species; years 3–6), declining significantly with the exclusion of native species by invasive grasses (years 9–14). Furthermore, the increasing dominance of invasive grasses was associated with a steady decline in the concentration of soil nitrogen and phosphorus per unit of accumulated carbon. These results demonstrate the importance of interdependent ecological and biogeochemical processes, and the role of soil–plant interactions in determining the success of restoration efforts. In contrast with previous but unsuccessful attempts to restore mined areas through nutrient application alone, iron-mediated stabilization of vegetation inputs favored the regeneration of a barren stable state that had persisted for over five decades since disturbance. The effectiveness of coupled organic matter and iron “fertilization,” combined with management of invasive species, has the possibility to enhance terrestrial carbon sequestration and accelerate the restoration of degraded lands, while addressing important challenges associated with urban waste disposal.

Key words: carbon sequestration; iron; land restoration; soil–plant interactions; stable isotopes; urban waste.

INTRODUCTION

Recently, we reported an unexpected accumulation of organic carbon in strip-mined areas undergoing restoration in central Brazil (Silva et al. 2013a). A number of processes that could have contributed to this large carbon accumulation were discussed in our previous study, but the specific mechanisms influencing the stabilization of carbon inputs from the vegetation remained unclear. Here we examine whether carbon accumulation was caused by the preferential stabilization of plant materials (in relation to biosolid amendment) into physically protected (occluded) soil aggregates, facilitated by the formation of iron-coordinated complexes. This hypothesis is based on previous theoretical work that describes the interplay between biological processes and chemical stabilization of organic materials, as an essential step for aggregate formation and accumulation of carbon in soils (Six et al. 2004, Melton et al. 2014, Parikh et al. 2014). Our empirical evaluation of such processes focused on testing this hypothesis while providing new information to guide management and restoration efforts in Brazil and elsewhere.
Biological aggregate formation has long been identified as an important component of soil carbon dynamics (Martin et al. 1955). Historical developments in aggregate theory have been extensively reviewed with a focus on agricultural soils (e.g., Six et al. 2004). Here we expand these concepts to the study of a young anthropo-sil silt-size aggregation (Tisdall and Oades 1982), with the critical different binding agents act at different stages of fungal hyphae and roots. When bound together by transient binding agents (e.g., complexes account for most of the plant carbon derived materials, first into micro- and then into macro-aggregates. Since iron oxides are the dominant binding agents in the original soil, and given that significantly will result from the microbial incorporation of plant-accumulation of chemically stable organic complexes developing anthrosols, we posit that a progressive focus on agricultural soils (e.g., Six et al. 2004). Here we aggregate theory have been extensively reviewed with a continuous arrival of seeds produced by the remaining trees and neighboring savanna vegetation, no sign of natural regeneration was observed. All sites are located within the limits of the Brazilian Federal District under homogeneous climatic and topographic conditions. The local climate is tropical with well-defined wet and dry seasons, Aw by Köppen’s classification. The mean temperature is 22.5°C with annual rainfall ranging from 1200 to 1600 mm and mostly distributed from November to March. Restoration activities initiated several decades after exploitation, using the same methods but taking place during a different year at each site, providing a chronosequence of time since restoration (Silva et al. 2013a). Restoration activities included the incorporation of biosolids (100 dry Mg/ha) as a common source of organic matter (50% carbon) and nutrients (N, 5.5%; P, 2%; K, 0.2%; Ca, 2.5%; Mg, 0.5%; S, 0.5%), produced through ferric ion precipitation during tertiary sewage waste treatment. Iron salts (Fe₂(SO₄)₃) are used as precipitating and stabilizing agents during the final stage of sewage treatment, so on average these biosolids have 45.3 ± 2.5 g/kg dry matter of highly reactive amorphous iron.

The recycling of sludge has been used throughout history and formally promoted for many years (e.g., EPA 1989). The term “biosolids” originally referred to colloidal cellular material produced during biological (secondary) treatment of wastewater, and was prevalent in water treatment literature from at least as early as the mid 1960s (Gates et al. 1967). In the early 1990s, however, “biosolids” was redefined and gained widespread use as a less objectionable term for treated sewage sludge deemed acceptable for land application, in order to emphasize the distinction between such material and untreated sludge (e.g., Gates et al. 1967, EPA 1989, Parker and Laha 2004). In this study, biosolids were added and incorporated by mechanical subsoiling, which then resulted in a homogeneous distribution of the applied material through the 0–20 cm layer (see Plate 1). Biosolid incorporation into exposed regoliths took place in different years, namely 1997, 2002, 2005, 2008, and 2011. Since our final measurements took place approximately six months after soil restoration in 2011, we were able to establish a site chronosequence as follows: 0.5, 3, 6, 9, and 14 years since restoration. Previous studies have used similar chronosequences to investigate autogenic plant succession (Baer et al. 2002, Simard et al. 2007) and soil carbon sequestration (Schelesinger 1990). This approach has proven particularly useful to study low-diversity systems of converging successional trajectories (Walker et al. 2010), such as degraded sites. Here we build upon this well-established framework, presenting and interpreting results as a function of time since restoration.

To ensure proper comparisons across sites, undisturbed soils and vegetation in the vicinity of each site served as reference for pre-disturbance conditions. Untreated exposed regoliths were also sampled at each site for comparison with treated ones. Consistent with
previous studies (Marques et al. 2004), untreated exposed regoliths were remarkably similar across sites, following an expected relation with the common bedrock, with texture ranging from silt loam to clay (sand, 190–310 g/kg; silt, 330–410 g/kg; clay, 210–550 g/kg), mostly composed of Si (580–710 g SiO2/kg soil), Al (140–210 g Al2O3/kg soil), and Fe (70–120 g Fe2O3/kg soil) and little to no vegetation cover or organic matter.

**Physical fractionation**

Soil samples from all sites were separated into four aggregate size fractions according to a modified method of Elliott (1986): light fraction (free particulate organic residue), macroaggregates (>250 μm), microaggregates (53–250 μm), and mineral particles (silt and clay; <53 μm). A 70-g subsample of soil was soaked for 5 min over a 250-μm sieve submerged in 1 cm of deionized water. The soil was subsequently sieved for 2 min by moving the sieve up and down with 50 repetitions. Macroaggregates remaining on the sieve were transferred into a pre-weighed container. The floating material was transferred to a pre-weighed container for isolation of the light fraction. The procedure was then repeated to separate microaggregates and mineral particles (i.e., silt and clay) by sieving the remaining soil and water through a 53-μm sieve. The mineral particles and water resultant from the 53-μm sieves were transferred to a final container. Free particulate organic carbon and aggregate fractions were oven-dried at 60°C and weighed. Total C associated with aggregates, mineral particles, and light fractions was determined on 10–20 mg subsamples by dry combustion gas chromatography. In this study, we present the C content associated with aggregates and light fractions. A description of all soil fractions, including the much smaller mineral fraction (<5% of the total soil C pool), can be found in our previous study (Silva et al. 2013a). In some cases water logging can lead to large increases in soil C, but here in situ measurements show a fast recovery of infiltration capacity shortly after the incorporation of biosolids and arrival of colonizing plants. Increases in infiltration rates from <5 to >10 cm/h are observed immediately after restoration (Appendix C) reaching ~30 cm/h on average by year 2 (Silva and Corrêa 2010). These rates are more than sufficient to prevent water logging during the rainy season, indicating that other factors must be considered to explain the formation of aggregates and carbon accumulation in the restored sites.

**Soil sampling and carbon determination**

After collection, soil samples were sieved (2 mm) and air dried. Soil organic carbon content was determined by dry combustion gas chromatography. Soil density (on average 1.3 g/cm3 in exposed regoliths and 1 g/cm3 in the restored sites) was used in the calculation of total soil carbon stocks, expressed as a fraction of soil mass at each depth. The reported accumulation of soil organic carbon over time is the average value of 15 randomly distributed plots at each site. The number of replicates was based on an a priori test of statistical power that
considered three predictors: time since restoration, soil depth, and vegetation cover, with an expected intermediate effect of time (site), measurable with moderate statistical power of 0.8 (Park 2010).

Isotopic approach

The isotopic method allows the quantification of different sources of carbon based on standard isotopic characterization of bulk soil organic carbon ($\delta^{13}C$) and the relative contribution of $C_3$- and $C_4$-derived carbon. The isotopic composition ($\delta^{13}C$) of biosolids applied during substrate treatment, colonizing plants, and resulting soil organic carbon was determined in all sites and multiple soil fractions by dry combustion gas chromatography, coupled with continuous-flow isotopic-ratio mass spectrometry. Across sites, the average $\delta^{13}C$ value of biosolids was determined to be $-27.1\% \pm 0.3\%$ (mean $\pm$ SE), which falls within the same range of carbon from $C_3$ plants. The average value for leaves of invasive grasses was $-12.2\% \pm 0.2\%$, which is a typical value for $C_4$ species. Using these signatures, a two-end member mixing model was used to quantify the effect of applied biosolids and invasive $C_4$ grasses, as distinct sources of carbon, using the following equation $C_4 = C_3 \cdot \frac{\delta_4 - \delta_3}{\delta_4 - \delta_3}$; where $C_4 = C_3 + C_4$ and represents the total amount of carbon in a given fraction at time $t$, $C_3$ is the amount of carbon derived from $C_3$ plants, $C_4$ is the amount of carbon derived from $C_4$ plants, $\delta_3$ is the $\delta^{13}C$ of the $C_3$ carbon, $\delta_4$ is the $\delta^{13}C$ value of the $C_3$ plant carbon, and $\delta_4$ is the $\delta^{13}C$ of $C_4$ carbon. The relative contribution of $C_3$ and $C_4$ carbon was expressed as percentage of total soil carbon in each pool.

Pyrophosphate-extractable iron

The amount of iron complexed with the accumulated plant-derived carbon was estimated by extraction with pyrophosphate. One gram of soil was shaken with 100 mL 0.1 mol/L tetrasodium pyrophosphate for 16 hours, followed by centrifugation for 30 min at 15 600× gravity to ensure removal of all fine iron colloids. The extracts were neutralized by a small addition of hydrochloric acid and the concentration of iron determined colorimetrically (Dominik and Kaupenjohann 2000). Dissolved organic carbon in pyrophosphate extracts was determined by UV-persulfate digestion (Phoenix 8000; Teledyne-Tekmar, Mason, Ohio, USA).

RESULTS

To provide an adequate context for the discussion of our new observations, we included here a figure modified from Silva et al. (2013a). This figure summarizes the two most important findings of that study: (1) a large accumulation of soil carbon almost five-fold higher than the levels typically found in undisturbed areas (Fig. 1A); and (2) a linear increase in the relative contribution of invasive $C_4$ grasses to the total soil carbon pool (Fig. 1B). Changes in the amount and source of soil carbon reflect shifts in plant community composition, summarized in Fig. 2. One of the remarkable findings of that study was a linear increase in the contribution of invasive ($C_4$) grasses to soil carbon stocks following a single application of iron-rich biosolids. Although several native $C_4$ grasses occur in the region, only exotic grasses of the genera *Brachiaria* and *Melinis* were found at the study sites. Combined, the incorporation of biosolids and subsequent plant colonization promoted soil development and accumulation of organic carbon to much higher levels than observed prior to disturbance.

The effect of invasive grasses was evident in the contribution of $C_4$ plant-derived material, which represented $>65\%$ of the total soil carbon accumulated over 14 years since the application of biosolids (Fig. 1B). The stability and distribution of soil carbon were briefly explored in our previous study (e.g., Silva et al. 2013a: Fig. 5), in which we isolated humin, humic acid, and fulvic acid fractions to show that chemically stable compounds in physically protected aggregates accounted for most of soil carbon accumulated over time. However, the role of iron-bound compounds in the formation of aggregates and whether this followed the theorized progression remained uncertain. Therefore, in the present study we examine the contribution of plants...
and biosolids to the formation of occluded carbon, hypothesizing that carbon accumulation was caused by the preferential stabilization of plant materials into occluded fractions, triggered by the formation of iron-coordinated compounds.

Our observations show that linear functions can be used to describe the relationship between occluded and total carbon accumulation. The slope of least square regressions indicates a much greater (2.6 times) accumulation of total soil carbon per unit of micro- relative to macroaggregate carbon (Fig. 3A). Total carbon accumulation is, nevertheless, mostly composed by the combination of both occluded pools (>80% of total carbon). This is observed particularly in the topsoil (Fig. 3A), with only minor contributions possible from the mineral fraction and undecomposed residues (i.e., light fraction). Confirming our expectations, the nonlinear trajectory of C$_4$ carbon accumulation into micro- to macroaggregates (Fig. 3B) reveals an equal initial distribution of these fractions (year 0.5) followed by a steep increase in the accumulation of microaggregate carbon derived from plants, subsequently translated into a proportionally greater accumulation of plant carbon into macroaggregates (micro/macro ratio <1 from year 3 onwards; Fig. 3B). As a result, the occluded carbon accumulation at year 14 originated mostly from invasive plants with total soil carbon being over threefold higher than that observed pre-disturbance, despite a significant decline from year 6 to year 14 (Fig. 1A) when invasive grasses excluded most native species (Fig. 2).

The first part of our hypothesis was therefore confirmed. The temporal trajectory of micro- and macroaggregate formation, combined with the contribution of C$_4$-derived carbon, show that decomposition and stabilization of plant inputs resulted in subsequent macroaggregate formation. Although no significant differences were observed with respect to carbon source immediately after the incorporation of biosolids, over 40% of microaggregates carbon was C$_4$-derived in year 3 (Appendix B). The contribution of C$_4$-derived carbon increased linearly thereafter to reach >70% of the total occluded carbon pool, showing a preferential stabilization of plant- in relation to biosolid-derived carbon. Along with changes in species composition (Appendix A), our observations also show a linear decline in nutrient (N and P) availability per unit of soil carbon accumulated as invasive grasses became more abundant (Fig. 4).

Confirming the second part of our hypothesis, we found an unequivocal association between occluded carbon pools and pyrophosphate-extractable iron (Fig. 5), which is indicative of a process of iron-mediated stabilization of organic compounds (Rasmussen et al. 2007, Song et al. 2013). It is well understood that iron oxides, in particular non-crystalline forms, bind organic particles in developing soils due to their high surface reactivity (Duiker et al. 2003). Although the indigenous soils that predominate in the region are rich in iron (and aluminum), these occur in the form of highly weathered nonreactive crystalline minerals. Under natural conditions accumulation of soil carbon typically does not exceed 2% of the soil mass, but the addition of amorphous iron in biosolids promoted a much higher accumulation of soil carbon originating from new plant inputs.
inputs (Figs. 1B and 3B). Combined, these findings suggest that the applied biosolid carbon was replaced with plant carbon over time, stabilized in iron-coordinated complexes, which served as nuclei for aggregate formation and soil development.

The relationship between pyrophosphate-extractable iron and total occluded carbon is described by a logarithm function that levels off at about 6 and 4 g C/100 g of topsoil and 10–20 cm depth soil, respectively (Fig. 5) achieved with an iron content about one order of magnitude lower (0.4–0.6 g/100 g soil). Importantly, these relationships show that microaggregates formation requires less iron than macroaggregate per unit of carbon accumulated, with both fractions representing a continuum of biological, chemical, and physical carbon stabilization. As discussed above, the seemingly small accumulation of iron-coordinated plant carbon as microaggregates, serves as nuclei for the formation of a much larger soil carbon pool (~140 Mg C/ha considering average levels of 7 g C/100 g of top soil 0–20 cm; Fig. 3A), in addition to the biosolids applied. The shape of these curves suggests, however, the existence of other factors limiting carbon accumulation beyond moderate levels of extractable iron. A progressive depletion in the pool of available nutrients could be the determinant of this threshold in aggregate formation.

DISCUSSION

Expected vs. observed results

Confirming our hypothesis, we verified that the process of carbon accumulation described in Silva et al. (2013a) can be best explained by the iron-mediated stabilization of plant materials into physically protected fractions. Soil aggregates accounted for >80% of the total soil carbon and were nucleated by iron-coordinated organic complexes formed shortly after biosolid addition (year 3). Biosolid amendment also provided an essential input of nutrients that prompted a process of autogenic succession leading to the continuous deposition of plant materials and soil development. A nonlinear trajectory confirmed the theorized progression from micro- to macroaggregate formation, with most occluded carbon originating from invasive C4 grasses. As invasive species became dominant, a strong linear increase in total soil carbon concentration followed the continuous formation of macroaggregates (years 6–9). However, significant declines in soil carbon accumulation were observed with the exclusion of native by invasive species (years 9–14), which coincided with a progressively lower concentration of N and P per unit of soil carbon accrued.

These results demonstrate the importance of considering interactions between autogenic succession, nutrient dynamics and soil development to predict the success of ecological restoration. Evidently, aggregate formation in developing soils is a dynamic process that differs from the simple linear progressions proposed by Tisdall and
Oades (1982). Elliott (1986) posited that because macroaggregates contain more labile and less microbiologically processed carbon than microaggregates, macroaggregate carbon is more easily lost upon changes in nutrients and vegetation inputs over time. Iron coordination can buffer this process through chemical stabilization and associated biological transformations as, for example, when the combination of glomalin (a protein produced by mycorrhizal fungi) and iron increases aggregate stability in hyphal networks (Rillig et al. 2002). Moreover, biomolecular P and carboxyl groups are known to facilitate bacterial adhesion to iron oxides (Parikh et al. 2014). Therefore, microaggregate formation would be expected to promote macroaggregate formation whenever organic matter becomes intimately associated with reactive forms of iron (Melton et al. 2014).

Here, two sets of observations identify the existence of this aggregate progression and the protective role of iron coordination: (1) the earlier formation of micro- in relation to macroaggregates; and (2) the increase in soil carbon concentration with increasing aggregate size class. Further support to these observations can be found in the progressive nutrient limitation across the chronosequence (Fig. 4). These observations are well described by early theoretical work (e.g., Greenland 1971), but also corroborate recent conceptual and experimental models that describe metal coordination as a key step in initial soil development, intersecting biological, chemical and physical processes of carbon stabilization (Six et al. 2004, Melton et al. 2014, Parikh et al. 2014).

Importantly, most of the anticipated progression in carbon content and aggregate formation happened quickly in the more biologically active soil layer (0–10 cm), but were later reflected in deeper layers showing an active vertical transport (Fig. 3B) and deeper soil development, likely associated with increased carbon input from roots. Accordingly, although delayed by a few years, the patterns described for the deeper soil layer reflect the same relationships between occluded and total carbon accumulation, nonlinear micro- to macroaggregate dynamics, and iron-mediated stabilization of plant carbon in protected fractions (Fig. 5). These findings indicate the existence of interdependent ecological and biogeochemical processes, encompassing complementary functions of energy accumulation and release (Holling 2001, Gunderson and Holling 2002), reflecting the general importance of soil–plant interactions.

The increasing nutritional constraints associated with the dominance of invasive species imply increased competition between plants and microorganisms, which could explain the threshold of occluded soil carbon accumulation (Fig. 5). Nutrient limitation is also considered a critical factor influencing successional trajectories and alternate stable states in natural vegetation gradients in the study region (Silva et al. 2013b, Franco et al. 2014; Paiva et al., in press). Here,

**Plate 1.** Sewage sludge application promotes ecological restoration and unprecedented carbon accumulation in previously barren mined areas of central Brazil. Photos: R. S. Correa.
causal links between nutrient availability and community composition could have influenced soil aggregate formation, leading to a decline in total carbon accumulation with the loss of species diversity (Figs. 1A and 2).

Broad significance

Although well established in the soils literature, the role of iron in stabilizing carbon remains to be formalized as a tool for ecological restoration. In our study, the addition of biosolids triggered carbon accumulation, amplifying the benefits of nutrient and organic matter addition with the stabilization of new inputs in iron-rich substrates. The synergistic effects of the amorphous iron applied as biosolids on biological, chemical and physical processes affecting soil development, were responsible for regenerating a barren stable state that had persisted for over five decades since disturbance.

Amorphous iron contrasts with crystalline forms abundant in the indigenous soil, being very reactive due to high surface area. These surfaces often account for most of the sorption and carbon stabilization, which include ternary (C-Fe oxide-clay) associations as well as the formation of unidentified bonds in soil organic complexes (Wagai and Mayer 2007). Soil carbon carries negative charges, whereas iron oxides are positively charged at the low pH (4–6) characteristic of these soils. Sludge application increases both soil pH and concentration of iron (Corrêa et al. 2010, 2012), so the addition of biosolid-borne amorphous iron yielded optimal conditions for carbon accumulation. This phenomenon has been recently observed in other environments (Tian et al. 2013) and has broad implications. Most of the developed and a large part of the developing world treats urban waste by generating biosolids through ferric ion precipitation. Our results suggest that these materials could be used to increase carbon sequestration in degraded soils, addressing important environmental challenges from increasing population growth, such as safe urban waste disposal and a growing need to increase terrestrial carbon sequestration to mitigate climate change.

About 24% of the global land area is covered by degrading or degraded soils, in which plant productivity have consistently declined over the past two decades (Bai et al. 2008). Mineralized tropical soils are notoriously difficult to restore. Previous studies have shown that the application of inorganic fertilizers in these areas, even when followed by planting of native species, fails to initiate autogenic succession or to promote accumulation of soil organic matter, ultimately leading to high plant mortality, perpetuating low energy steady states (Martins et al. 2004, Starr et al. 2012). Our findings contrast with previous unsuccessful efforts and show that coupled organic matter and iron amendment is a viable approach to promote ecological restoration and soil development. Secondary sludge would likely contain similar amounts of many of the nutrients found in the tertiary sludge used here. However, it is rare that such material would be applied to land, due to difficulty in handling (large liquid content) and environmental regulations. Furthermore, while the nutrient content may be similar, tertiary sludge treated with chemical coagulants contains a large amount of iron and/or aluminum (oxy)hydroxides, and is therefore physically and chemically superior in relation to other waste materials.

In other biomes, long-term carbon accumulation has been shown to override annual fluctuations (Doane and Horwath 2004) and persist due to association between iron and organic matter (Kögel-Knabner et al. 2008). Consistent with our observations, Tian et al. (2013) found a “remarkable increase” in stability of organic carbon under long-term biosolid application, which was dependent not only on iron but also on nutrient dynamics. This reinforces the critical role of plant succession in regulating the availability of limiting resources and the need to manage invasive species in long-term restoration projects, even when the emphasis is on increasing soil carbon sequestration. Environmental controls of carbon stability determine thresholds beyond which the mineral fractions of a soil saturate and additional carbon inputs would only accumulate into labile pools (Gulde et al. 2008, Kögel-Knabner et al. 2008). In temperate systems, saturation levels are similar to the values reported here (180 Mg C ha⁻¹ yr⁻¹ vs. 140 Mg C ha⁻¹ yr⁻¹ in this study). This demonstrates that increasing terrestrial carbon sequestration is possible, regardless of environmental controls limiting indigenous levels, by harnessing ecological and biogeochemical process to accelerate the restoration of degraded lands.

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