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Initial Recovery of Organic Matter of a Grass-Covered Constructed Soil after Coal Mining

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ABSTRACT: Revegetation is essential to improve the quality of constructed soils. This study investigated effects of perennial grasses on total organic carbon (TOC) stock, organic matter (OM) fractions and on quality of a recently constructed soil, after coal mining. Soil samples were collected from the 0.00-0.03 m layer two years after the beginning of the experiment. The treatments consisted of *Cynodon dactylon* cv vaquero (T1); *Urochloa brizantha* (T2); *Panicum maximun* (T3); *Urochloa humidicola* (T4); *Hemarthria altissima* (T5); *Cynodon dactylon* cv tifton (T6); bare constructed soil (T8); and natural soil (T9). The treatments with grass species increased the TOC stock by 57 % and increased the OM lability in comparison with T8. Higher C accumulation in the coarse and free light fractions and a higher C management index were observed in T2 and T3, indicating greater suitability of these species for the initial recovery of OM of the constructed soil.

Keywords: degraded areas, physical fractionation, carbon management index, laser-induced fluorescence.

INTRODUCTION

The greatest coal reserves of Brazil, of about 12.3 billion tons, are located in Candiota, Rio Grande do Sul (RS) (Santucci, 2009). This is the municipality with the largest coal seam of the country (Mina de Candiota), where deposits of around 1 billion tons can be surface mined (CRM, 2015). This mine is operated by the Companhia Riograndense de Mineração (CRM), and supplies the thermoelectric power plant Presidente Médici. To adjust the expansion of the power plant from a capacity of 446 to 796 MW in 2010, the CRM doubled its output capacity to an annual supply of the power plant of about 3.3 million tons of coal (CRM, 2015). Although coal is essential for power generation and other processes, there is a threat in this expansion of the mining area, to cause a parallel expansion of mining-degraded land.

The first step of opening a mine is the removal and storage of surface soil horizons, for later use in the topographic reclamation of the mined area, originating the so-called "constructed soils". Due to the movement of the surface horizons during soil withdrawal (pre-extraction of coal), storage and subsequent deposition as surface layer of the constructed soil, these soils usually have high degradation and compaction levels, low fertility and a particularly poor quality of organic matter (OM), hampering the recovery of soil quality (Corrêa, 2009; Stumpf et al., 2014a; Leal et al., 2015a; Miola et al., 2015).

The revegetation of constructed soils is essential for the environmental reintegration of the reclaimed area and to improve soil quality, protecting the soil surface against erosion, breaking compacted layers, stabilizing the soil, and increasing the OM content (Müller et al., 2001; Lima et al., 2012). Higher total organic carbon (TOC) contents in the 0.00-0.10 m layer of a soil constructed after coal mining covered with grasses for 9 and 30 years (19.2 and 18.9 g kg^{-1}, respectively), in comparison with soil covered for one year (5.78 g kg^{-1}), were reported by Chatterjee et al. (2009). These data corroborate those of Ganjegunte et al. (2009), who found a higher TOC stock in the 0.00-0.30 m layer of a soil constructed after mining and covered predominantly with grass for 26 years (38.4 Mg ha^{-1}), compared to a soil covered with vegetation for less than one year (24.1 Mg ha^{-1}).

As a result of the different mechanisms protecting different OM fractions against degradation, labile fractions are usually more sensitive to changes in crop and soil management than the unfractionated OM (Conceição et al., 2008; Guimarães et al., 2013). The physical fractionation method has been preferentially used instead of chemical fractionation, to study OM cycling rates in soils under agricultural cultivation and for identifying more favorable plant cultivation and/or soil management systems to improve the soil quality (Rosa et al., 2011; Conceição et al., 2014; Mafra et al., 2015).

The classical method of physical particle-size fractionation of OM, basically separates OM in a particulate fraction (labile, > 0.053 mm) and a fraction associated with soil minerals (non-labile, <0.053 mm) (Cambardella and Elliot, 1992). This method is easily applicable and widely used in the study of OM. With regard to the physical density fractionation, a higher efficiency of sodium polytungstate solution (SPT) than of sodium iodide solution (NaI) was reported in the recovery of light OM fractions (Conceição et al., 2014; 2015). According to the authors, the interaction of iodide ions with organic compounds contained in these fractions probably underestimates the C contained therein. With a view to the particle size of the samples used for density fractionation, Tomazi et al. (2011) observed a lower recovery of occluded light fractions when using samples sieved through 2.00 mm instead of 9.5 mm mesh. According to the authors, the use of samples sieved through the former underestimates the C content protected physically within aggregates. Therefore, the use of SPT solution and samples sieved through 9.5 mm mesh are considered more appropriate for the density fractionation of OM.

The carbon management index (CMI) can be calculated from the particle-size or density fractions of OM. This index reflects the impact of different management systems not only on the OM content but also the quality, by a comparative assessment (between treatments) of higher or lower OM lability; the higher the CMI, the better the soil quality (Conceição et al., 2014).

The CMI has been estimated from particle-size fractions (Vieira et al., 2007; Schiavo et al., 2011; Guimarães et al., 2014; Zhao et al., 2014). Recently, Conceição et al. (2014) found a high correlation (r = 0.92; p<0.01) between CMI estimated from these fractions and from density fractions (separated with SPT solution). The authors observed that the density-based CMI was greater in discriminating crop and soil management systems. According to the authors, this was due to the higher efficiency of the density method in the recovery of the labile OM fraction. These data corroborate results of Leal et al. (2015b). In a soil constructed after coal mining and covered with grasses for six years, these authors found a high correlation (r = 0.95; p<0.05) between CMI estimated from particle-size and from density (separated with SPT) OM fractions, and greater sensitivity of the density-based CMI to distinguish the most appropriate plant species for the recovery of the quality of the constructed soil.

To complement the information on the quantitative and qualitative status of OM provided by the physical fractionation and the CMI, spectroscopic techniques for the chemical characterization of OM can be used. For this purpose, the laser-induced fluorescence (LIF) spectroscopy is a non-destructive technique used to determine the degree of OM humification in whole soil samples, requiring no sample treatment nor extraction/separation of OM fractions (Milori et al., 2006). To normalize the fluorescence signal, Milori et al. (2006) proposed the LIF index (I_{LIF}), which divides the fluorescence emission spectrum by the C content per sample, making a comparison of different soil samples (treatments) possible. Soils under conservation tillage practices and with high organic residue input tend to have lower I_{LIF} than soils under conventional tillage, where the OM protected in aggregates is exposed to degradation. Additionally, the lower input of fresh organic material in the soil alters the OM composition, making it proportionally less labile (Milori et al., 2006; Favoretto et al., 2008).

In recently constructed soils, where OM consists predominantly of less labile material, I_{LIF} is expected to decrease over the first years of revegetation, due to the reduced soil disturbance, the protection of the soil surface against erosion, the incorporation of plant residues into the soil, and the OM protection within aggregates (Leal et al., 2015b). Studies on OM including spectroscopic techniques associated with fractionation methods are common in soils under agricultural cultivation (Roscoe et al., 2001; Bayer et al., 2002; Dieckow et al., 2005; Boeni et al., 2014). However, few studies use these techniques to evaluate the quality of drastically degraded soils, as in the case of constructed soils (Schiavo et al., 2007a,b; Leal et al., 2015a,b). In this context, the CMI estimated from density fractions as well as LIF spectroscopy are promising techniques for an early identification of the most appropriate crop systems to improve the quality of constructed soils.

Thus, it is hypothesized that the combined use of density-based CMI and labile OM with I_{LIF} as soil indicators sensitive to the cultivation of different plant species allows an identification of the most adequate cover crop species for the initial revegetation and quality improvement of newly constructed soils. In this sense, the purpose of this study was to evaluate the effect of different grasses on the OM density fractions, estimate the CMI there of and qualitatively assess the OM by LIF, in order to detect the most appropriate grass(es) to improve the quality of the constructed soil in Candiota, RS.

MATERIALS AND METHODS

Study area and soil sampling

The experimental area (31° 33′ 55,5″ S and 53° 43′ 30,6″ W, at 230 m asl), in the region of Campanha of RS state, is a coal mining area that belongs to the Companhia Riograndense de Mineração (CRM), in the municipality of Candiota.

The climate was classified as Cfa (humid subtropical), according to the classification of Köppen system. The natural soil prior to mining was classified as *Argissolo Vermelho Eutrófico típico* (Embrapa, 2006), a Rhodic Lixisol (IUSS, 2014), and the 0.00-0.05 m layer has a loamy texture (Table 1).

Prior to coal extraction, the surface horizons of the Lixisol were removed and stored (Figure 1a). Thereafter, the coal layer of interest was exposed and coal was extracted (Figure 1b). Soon after, a topographic recovery of the area was performed on the overburden piles. Finally, the mixture of Lixisol surface horizons (predominantly B horizon) was deposited on the surface (Figure 1c), forming the “constructed soil”. This soil was constructed in 2003 and the above-mentioned horizon mixture formed a 0.30-0.40 m

Table 1. Particle size distribution, textural class and bulk density (BD) in the 0.00-0.05 m layer of a constructed soil covered with different grass species, a bare constructed soil and a natural soil (unmined, with native vegetation)

Treatment	Clay	Sand	Silt	Textural class	BD
	g kg^{-1}				Mg m^{-3}
T1 – Vaquero	468.0	312.8	219.1	Clay	1.48
T2 – Brizantha	455.5	318.9	225.6	Clay	1.39
T3 – Tanzania	475.4	307.0	217.6	Clay	1.43
T4 – Humidicola	479.6	313.3	207.1	Clay	1.41
T5 – Hemarthria	463.9	314.3	221.8	Clay	1.42
T6 – Tifton	461.4	315.8	222.8	Clay	1.41
T8 – Bare constructed soil	456.7	311.7	231.5	Clay	1.46
T9 – Natural soil	227.2	483.6	289.1	Loam	1.47

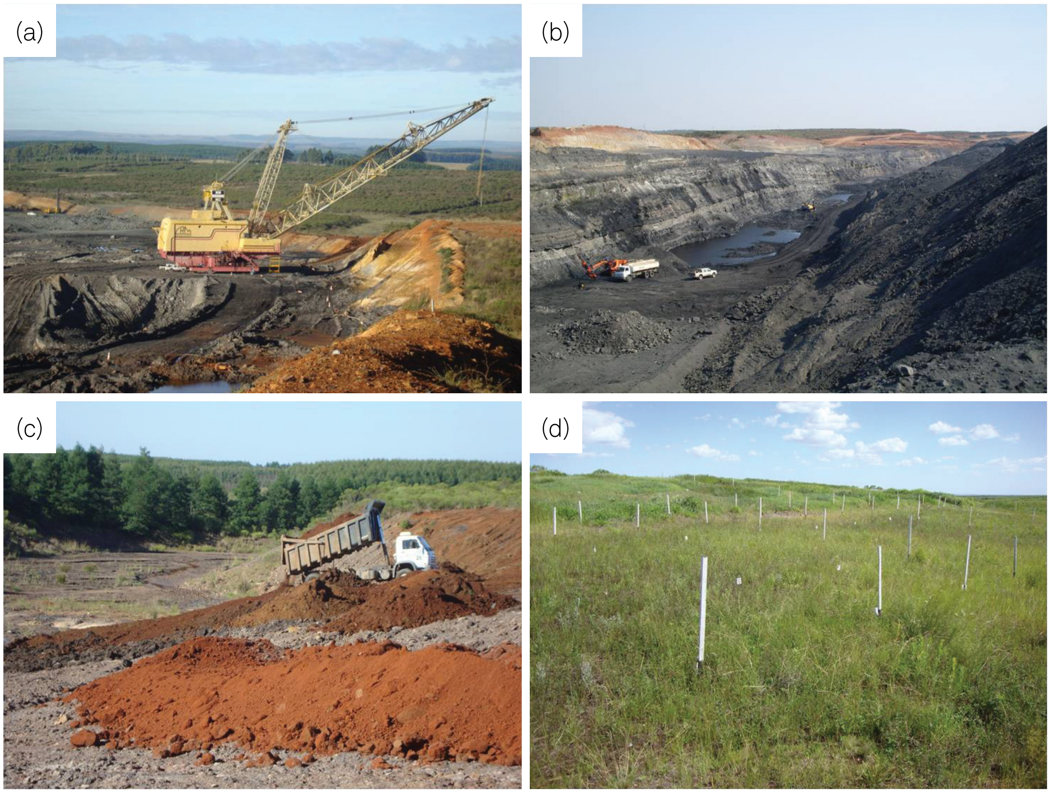

Figure 1. Simplified sequence of the steps from coal mining to the start of the experiment, as follows: (a) removal of surface soil horizons; (b) exposure and extraction of coal layers of interest; (c) distribution of a mixture of Lixisol horizons (previously removed) on the topographically reclaimed area, forming constructed soil; and (d) performance of the experiment on the constructed soil.

thick layer, comprising the topsoil. The textural class of the 0.00-0.05 m layer of the constructed soil is clayey (mean clay content of 466 g kg^{-1}) (Table 1).

The experiment was installed in September/October 2007 on the constructed soil (Figure 1d) and was designed for long duration. The data of the chemical properties of the constructed soil can be found in the study of Stumpf et al. (2014b). The experiment was arranged in randomized blocks with four replications, each of which was represented by a 20 m^2 (4 × 5 m) plot. The treatments evaluated in this experiment consisted of the following perennial grass species: T1 – cv Vaquero [*Cynodon dactylon* (L.) Pers]; T2 – Brizantha [*Urochloa brizantha* (Hochst.) Stapf]; T3 - Tanzania (*Panicum maximum* Jac); T4 – Humidicola [*Urochloa humidicola* (Rendle) Schweickt]; T5 – Hemarthria [*Hemarthria altissima* (Poir.) Stapf & C. E. Hubbard] and T6 – cv Tifton [*Cynodon dactylon* (L.) Pers.]. Treatment T7, originally composed of *Hemarthria altissima* + *Cynodon dactylon* + spontaneous vegetation, was not evaluated in this study because the plants did not survive, probably due to the high degradation level of the constructed soil.

In the beginning of the experiment, the soil was chiseled to a depth of 0.10-0.15 m. After chiseling, lime (10.4 Mg ha^{-1}) with a total relative neutralizing power of 100 % and a fertilizer mixture (900 kg ha^{-1} N-P-K, 5-20-20) were applied. Every spring (October/November), N fertilization was applied in the form of ammonium sulfate (40 kg ha^{-1} N) and undesired plants were removed by hand weeding.

About two years after the beginning of the experiment, in September 2009, soil samples were collected from the 0.00-0.03 m layer, with minimal disturbance to preserve the aggregate structure, and air-dried. Undisturbed samples were collected in steel cylinders (height 3.00 cm, diameter 4.85 cm) from the 0.00-0.05 m layer, to determine bulk density (BD).

For comparison, samples were also collected in areas adjacent to the experiment: bare constructed soil (T8) and natural soil (unmined, Lixisol) under predominantly shrubby native vegetation (T9).

Particle-size fractionation of OM

The particle-size fractionation was performed as described by Cambardella and Elliott (1992). In glass flasks, 20 g of soil (sieved to 2.00 mm) were blended with 60 mL of 5.0 g L^{-1} sodium hexametaphosphate. The flasks were placed in a shaker and shaken horizontally (150 oscillations min^{-1}) for 15 h. Then the suspension was washed with distilled water on a sieve (mesh 0.053 mm). The material retained on the sieve, corresponding to the coarse fraction, was dried at 50 °C and its C content (CCF) determined. The contents of total nitrogen (TN), CCF and TOC were determined in an elemental analyzer (TruSpec-CHN). The C content of the fraction that passed through the sieve, the mineral-associated C (MAC), was calculated as the difference between the TOC and CCF content (MAC = TOC – CCF).

Density fractionation of OM

The density fractions were obtained according to the method described by Conceição et al. (2008). First, 10 g of soil were weighed according to the proportion of the aggregate size groups (<2.00 mm and aggregates between 9.52 and 2.00 mm). The SPT solution (80 mL, density 2.00 Mg m^{-3}) was filled in centrifuge tubes containing the proportionally weighed samples. After shaking the suspension by hand, the tubes were centrifuged (2,000 *g* for 1 h). The supernatant was vacuum-filtered through a glass-fiber filter to separate the free light fraction (FLF). The SPT solution was added again to the remaining material in the tubes, and this suspension was subjected to ultrasonic dispersion. The energy required in the application of ultrasound for the complete dispersion of the soil samples was previously determined for the different situations of the experiment: covered constructed soil (T1, T2, T3, T4, T5 and T6) – 430 J mL^{-1}; bare constructed soil (T8) – 360 J mL^{-1}; and natural soil (T9) – 576 J mL^{-1}. The method to calculate energy dispersion was described by Leal (2011).

The dispersed suspension was centrifuged and filtered to separate the occluded light fraction (OLF) retained on the filter, and the heavy fraction (HF) remaining in the centrifuge tube. The FLF and OLF fractions were oven-dried at 50 °C, ground in a mortar and the C content was determined with an elemental analyzer (TruSpec-CHN). The C content of the HF was computed as the difference between TOC and light fractions {HF = [TOC – (FLF + OLF)]}. The stocks of TOC, TN and C in OM fractions were calculated based on the C content (total or fractions) and TN and BD (Table 1).

Carbon management index (CMI)

The density fractions were used to calculate CMI (Equation 1), where the C stock in FLF was used as labile C and the C stock in HF as non-labile C. To estimate the CMI, the bare constructed soil (T8) was used as control treatment, since the objective was to observe the improvement in the constructed soil quality resulting from the implantation of cover species.

$$CMI = CPI \times LI \times 100 \qquad \text{Eq. 1}$$

where

CPI (Carbon Pool Index) = TOC stock of treatment/TOC stock of control treatment (T8);

LI (Lability Index) = L of treatment/L of control treatment (T8); where L is calculated as:

L (Lability) = labile C stock (FLF)/non-labile C stock (HF).

Laser-induced fluorescence (LIF) spectroscopy

Composite soil samples (consisting of the four field replications), sieved (mesh 9.52 mm) and ground in an agate mortar, were used for the LIF analysis. These samples were pressed by a manual preparation system, forming pellets (thickness 2 cm, diameter 1 cm), which were analyzed in a portable LIF system.

The index of laser-induced fluorescence (I_{FIL}) was calculated according to the method described by Milori et al. (2006), as the ratio of the area under the fluorescence emission spectrum curve (AFC) between 475 and 660 nm and the TOC content of the sample. Positive linear correlation (R^2= 0.82, p<0.01) between AFC and TOC was observed (data not shown). The values of AFC, TOC and the LIF emission spectra were reported by Leal (2011).

Statistical analysis

No outliers were detected and the variables: TOC, TN, CCF, MAC, FLF, OLF, and HF had a normal distribution by the Shapiro-Wilk W test. The data were subjected to analysis of variance and Duncan test, at 5 % probability. The treatments T8 and T9 were not included in the statistical analysis for not being part of the experimental design and were therefore only used as parameters, for monitoring the recovery process of the constructed soil. Pearson's correlation coefficient (r) was calculated as the correlation between I_{LIF} and CMI.

RESULTS AND DISCUSSION

Effect of cover crops on TOC and OM fractions

In the treatments with cover species, the TOC stocks ranged from 4.09 to 5.44 Mg ha^{-1} (Table 2), with no significant differences.

Compared to the bare constructed soil, cover species increased the TOC stock on average by 57 %, indicating, although at an early stage, a tendency towards a partial recovery of the TOC stock. This reflects the good adaptability of the grass species to the unfavorable growth conditions of this constructed soil and to the commonly adverse climate features in the experimental area, with frequent frost in the winter and high temperatures and little rainfall in the summer. These data are consistent with those of Leal et al. (2015a), in that the 0.00-0.03 m layer of a soil constructed after coal mining and covered with grasses for six years contained a 79 % higher TOC stock than a bare constructed soil.

Despite these effects, this increase must not be interpreted as a total recovery of soil OM. The TOC stocks in the cover species treatments accounted on average for only 38 % of the TOC stock of the natural soil. This can be ascribed to the high degradation degree of the constructed soil, which reduced these values drastically and to the short time between the planting of the cover species and soil sampling, two years later. The experiment was designed as a long-term project and the soil will not be tilled because the planted species are perennial, so that the TOC stocks are expected to increase gradually in the surface layer over time. However, due to soil disturbance during construction, differentiating it from the natural soil, cover crops are likely to be required for decades until the TOC stocks of the constructed soil reach the level of those of natural soil. These results corroborate findings of Anderson et al. (2008). The authors observed that the TOC stock in of 0.00-0.05 m layer of a constructed soil after coal mining and covered with grasses for 11 years contained only 30 % of the natural soil TOC stock (covered with shrubs and grasses).

The TN stocks in the cover species treatments exceeded those of bare constructed soil by 23 % on average. Among the cover species treatments, the TN stock was highest in T3 (0.36 Mg ha^{-1}), which did not differ from T2 (0.31 Mg ha^{-1}) (Table 2). However, considering that the experiment is in an early stage and that the differences in TN stocks are, in practice, very subtle, further analysis will be needed to identify the plant species with greatest potential to increase the TN stocks consistently. On average, the TN stock of cover crop treatments was 58 % of that of natural soil, indicating a partial recovery of TN stocks.

Although no difference was detected between TOC stocks of the cover species treatments, the CCF fraction was sensitive to their respective effects. This fraction is mainly composed of plant residues in different stages of change and its main protection mechanism is the biochemical recalcitrance of the organic molecules. Therefore, the CCF fraction is often more sensitive to changes in crop and soil management systems than TOC, where other mechanisms such as physical occlusion in aggregates and interaction with minerals

Table 2. Stocks of total organic carbon (TOC), total nitrogen (TN), carbon stock in the coarse fraction (CCF), mineral-associated carbon (MAC), and CCF/TOC and MAC/TOC ratios in the 0.00-0.03 m layer of a constructed soil covered with different grass species, a bare constructed soil and a natural soil (unmined, with native vegetation)

Treatment	TOC	TN	CCF	MAC	CCF/TOC	MAC/TOC
	Mg ha^{-1}				%	
T1 – Vaquero	4.47 a	0.286 bc	1.52 b	2.95 a	33	67
T2 – Brizantha	5.07 a	0.313 ab	2.27 a	2.80 a	44	56
T3 – Tanzania	5.44 a	0.361 a	1.73 ab	3.71 a	32	68
T4 – Humidicola	4.09 a	0.248 c	1.02 b	3.07 a	25	75
T5 – Hemarthria	4.32 a	0.279 bc	1.26 b	3.06 a	29	71
T6 – Tifton	4.26 a	0.282 bc	1.41 b	2.85 a	33	67
Mean T1-T6[(1)]	4.61	0.294	1.54	3.07	33	67
T8 – Bare constructed soil	2.93	0.239	0.82	2.11	27	73
T9 – Natural soil	12.20	0.510	4.27	7.92	35	65

Means followed by the same letter in a column do not differ statistically by the Duncan test ($p<0.05$). [(1)] Mean of cover species treatments: T1, T2, T3, T4, T5, and T6.

contribute to OM protection (Bayer et al., 2004). The higher sensitivity of the CCF fraction than the TOC to distinguish the effect of different crop and/or soil tillage systems was also observed by other authors (Schiavo et al., 2011; Conceição et al., 2014).

On average, the CCF stock in the cover species treatments was 87 % higher than that of bare constructed soil, indicating that the grasses were efficient for the initial recovery of CCF stocks. Growing Brizantha (T2) resulted in the highest CCF stock (2.27 Mg ha^{-1}) (Table 2), which did not differ from Tanzania (T3). In general, these species have vigorous root systems, drought tolerance, wide adaptation to different conditions of climate and soil fertility, and above all, tend to produce high dry matter yields, with a better development than other cover crop species (Soares Filho et al., 2002; Cano et al., 2004; Santos and Costa, 2006; Barducci et al., 2009). The higher suitability of these species compared to other grasses to recover the quality of the constructed soil in Candiota, particularly concerning the chemical and physical soil properties, was recently reported in the literature (Lima et al., 2012; Reis et al., 2014; Stumpf et al., 2014a,b; Miola et al., 2015).

The greater potential of T2 and T3, mainly of T2, in increasing the labile OM in the soil, is corroborated by the higher ratio CCF/TOC (44 %) observed in this treatment in comparison to the other treatments with cover crops (varying from 25 to 35 %). The C increase in this fraction is essential for the recovery of soil quality, since labile OM can serve as energy source for the soil biota and as nutrient source for plants (Dieckow, 2003; Leite et al., 2010).

Despite the increase in the CCF stock induced by the grasses compared to bare soil, on average, it accounted for only 36 % of that of natural soil. These data indicate the high degradation degree of constructed soil and suggest the need for long-term grass cultivation, possibly associated with other species in the future, for a full recovery of the CCF stock.

In the cover species treatments, the MAC stocks ranged from 2.80 to 3.71 Mg ha^{-1} (Table 2), but did not differ statistically between the cover species used for revegetation. The MAC fraction consists of less labile organic material, subject to different protection mechanisms involved in OM stabilization, including protection within microaggregates, in this case <0.053 mm (Carter and Stewart, 1996; Bayer et al., 2004) and interactions with the surface of mineral particles (electrostatic attraction – outer sphere complex, sorption by cation bridge – internal sphere complex, protonation, hydrophobic interactions), forming organo-mineral complexes (Dick et al., 2009). These factors make the MAC fraction less sensitive to different soil management/crop systems and more stable in comparison to the CCF fraction (Bayer et al., 2004; Leal et al., 2015b). Therefore, the MAC fraction often contains higher TOC proportions, playing a key role in maintaining the soil TOC stocks. In fact, in all treatments the MAC/TOC ratio (56-75 %) was higher than the CCF/TOC ratio (25-44 %), reinforcing the importance of MAC for the TOC stock.

On average, the MAC stock was 45 % higher in cover species treatments than in bare soil. Since the MAC stock depends on C derived from CCF fraction and as this in turn depends on the organic residues added to the soil, it was expected that the MAC stock of the bare soil would be lower than that of the other treatments. In addition, without cover crops, the soil is more exposed to erosion, accelerating OM losses, including losses from the MAC fraction.

Although the covered constructed soil has a higher percentage of microaggregates (Stumpf et al., 2014b) and higher clay content compared to natural soil (Table 1), the MAC stock of covered constructed soil represented, on average, only 39 % of that of natural soil. This can be attributed to the constant addition of plant residues to the natural soil over the years and its low disturbance, leading to preservation of non-occluded OM associated with silt and clay fractions and to greater stability and protection of OM occluded in microaggregates (Six et al., 1998; Bayer e al., 2004; Virto et al., 2008). The higher C stock in the OLF and HF fractions of natural soil in comparison with bare or covered constructed soils (Table 3) reinforces this interpretation.

The FLF was also sensitive to different plant species used for revegetation of the constructed soil. The biochemical resistance of the organic molecules is the main protection mechanism of the FLF, resulting in a greater lability of this fraction than of the OLF and HF fractions, which have a higher stability due to additional protection mechanisms (Christensen, 2001).

All cover species increased the C stock in FLF compared to the bare constructed soil; on average this increase was 100 %. The increase in FLF stocks in constructed soils is important for improving the soil quality, for serving as a nutrient source for plants and for supplying C to the other OM fractions. The C stock in FLF was highest (0.96 Mg ha^{-1}) when Brizantha was grown, and did not differ statistically from the Tanzania treatment (0.82 Mg ha^{-1}) (Table 3). These data indicate the greater potential of these species for the input of labile OM into the soil and their greater adaptability to degraded soil. However, none of the treatments reached the C stock in the FLF of natural soil (2.65 Mg ha^{-1}) (Table 3). On average, the cover crop treatments accounted for only 24 % of the stock in natural soil, indicating that it will probably take years/decades of cultivation until the C stock in the FLF of covered constructed soil reaches that of natural soil. Results in this direction were observed by Leal et al. (2015b) in the 0.00-0.03 m layer of a constructed soil covered with grasses after coal mining. After six years of cover crops cultivation, the authors observed that the C stocks in the FLF of covered constructed soil were on average 237 % higher than those in bare constructed soil, but represented only 39 % of the stock in the natural soil.

In this same experiment, Stumpf et al. (2014b) evaluated the effect of different grass species on soil physical properties. After two years, the authors found that, compared to other grasses, Brizantha (T2) caused a significant increase in mean weight diameter (MWD) of aggregates and percentage of macroaggregates in the 0.00-0 05 m layer of the constructed soil. However, the improvement of these soil physical properties induced no significant differences in C stocks in the OLF, which ranged from 0.37 to 1.00 Mg ha^{-1} (Table 3). Due to the effect of protection mechanisms acting on OLF (biochemical recalcitrance of organic molecules and the physical protection within aggregates), this fraction is often less sensitive to changes in the soil management/crop systems than FLF (Sollins et al., 1996).

Usually, a positive correlation between the C stock in OLF and MWD is observed, since the organic material provides nuclei for aggregate formation and the decomposition

Table 3. Carbon stocks in the free light fraction (FLF), occluded light fraction (OLF), heavy fraction (HF), FLF/TOC, OLF/TOC and HF/TOC ratios, carbon pool index (CPI), carbon lability (L), lability index (LI) and carbon management index (CMI) in the 0.00-0.03 m layer of a constructed soil covered with different grass species, a bare constructed soil and a natural soil (unmined, with native vegetation)

Treatment	FLF	OLF	HF	FLF/TOC	OLF/TOC	HF/TOC	CPI	L	LI	CMI
	Mg ha^{-1}			%						
T1 – Vaquero	0.58 bc	0.54 a	3.35 a	13	12	75	1.53	0.18	1.48	219
T2 – Brizantha	0.96 a	1.00 a	3.12 a	19	19	62	1.73	0.32	2.63	453
T3 – Tanzania	0.82 ab	0.82 a	3.80 a	15	15	70	1.86	0.22	1.79	332
T4 – Humidicola	0.46 c	0.37 a	3.26 a	11	9	81	1.40	0.14	1.15	162
T5 – Hemarthria	0.38 c	0.61 a	3.34 a	9	14	78	1.48	0.11	0.92	137
T6 – Tifton	0.53 bc	0.62 a	3.10 a	12	15	73	1.45	0.18	1.49	216
Mean T1 – T6[1]	0.62	0.66	3.33	13	14	73	1.58	0.19	1.58	253
T8 – Bare constructed soil[2]	0.31	0.69	1.88	11	2	87	1.00	0.12	1.00	100
T9 – Natural soil	2.65	1.87	5.50	22	15	63	4.16	0.36	2.94	1,204

Means followed by the same letter in a column do not differ statistically by the Duncan test ($p<0.05$). [1] Mean of cover species treatments: T1, T2, T3, T4, T5, and T6. [2] Control treatment for CMI calculation.

products serve as aggregate stabilizers, reducing the access of microorganisms and their enzymes to organic compounds (Conceição, 2006). Despite the differentiated effect of plant species on soil physical properties, the short experimental duration, associated with the high degradation and compaction degree of the constructed soil, which slow down the process of soil recovery and aggregation, probably prevented a differentiated effect of cover crops on C accumulation in OLF.

These results differ from those reported by Leal et al. (2015b) in a 6-year experiment, which evaluated the effect of grass species on OLF in the 0.00-0.03 m layer of a soil constructed after coal mining. The authors observed that some species induced a greater C accumulation in OLF than others. The longer experiment duration (6 years) than in this study (2 years) probably allowed the differentiation of the cover species.

The mean C stock in OLF of cover species treatments (0.66 Mg ha^{-1}) was similar to that of bare constructed soil (0.69 Mg ha^{-1}) (Table 3). The short experimental duration and the high degradation level of constructed soil, hampering the land reclamation process, probably contributed to prevent a higher C stock in OLF in the covered constructed soil. These factors may also explain why OLF stocks of treatments with cover crops represented only 35 % of the stock in the natural soil.

The C stocks in the HF of the cover species treatments ranged from 3.10 to 3.80 Mg ha^{-1}, and significant differences were not observed (Table 3). Compared to bare soil, the cover species treatments increased the HF stock by 77 % on average. However, these stocks accounted for only 61 % of the stock in the natural soil. These data agree with those of Leite et al. (2015), who found a low C content in the labile and non-labile OM of the 0.00-0.05 m layer of an Acrisol covered with *Jatropha curcas* for one year, after a long period of degradation by mining and uncontrolled burning and grazing. The HF is considered the OM density fraction with least sensitivity to changes in the soil management/crop systems, because aside from the biochemical recalcitrance of organic molecules and physical protection, the association of OM with soil minerals acts as a protection mechanism. Therefore, the proportion of C is normally higher in the HF than in the FLF and OLF fractions. In this study, the ranges of the FLF/TOC, OLF/TOC and HF/TOC ratios were 9-22 %, 2-19 % and 62-87 % (Table 3), respectively, confirming the greater representation of HF than of the light fractions and the relevance of HF for the maintenance of the total OM stocks.

OM lability and CMI

All cover species treatments increased the CPI, L and LI by 58, 60 and 58 % (on average) respectively, in comparison with the control (bare constructed soil), except for the Hemarthria treatment (T5), which resulted in lower L and LI (Table 3). Although the TOC stock in T5 did not differ from the other treatments, L and LI data indicate the weaker capacity of Hemarthria to increase the labile OM portion.

On average, the values of CPI, L and LI observed in the cover crop treatments accounted for 38, 53 and 54 %, respectively, of the values of natural soil. Despite the cover species planted on the constructed soil, increasing the labile organic material in the soil, most of the OM was probably reminiscent from the natural soil (coal pre-extraction), especially the non-labile, represented by the HF. On average, the HF/TOC ratio in the cover crop treatments was 73 %, while in natural soil this value was 63 % (Table 3), indicating the high contribution of HF especially to OM of covered constructed soil.

The CMI values in cover species treatments were 153 % higher (on average) than those of bare constructed soil, indicating the positive effect of the grasses on quality of the constructed soil. Brizantha resulted in the highest CMI (453) and Hemarthria in the lowest (137). The best adaptation of Brizantha to the constructed soil and to the characteristic regional climate of the experimental area may have resulted in the greater input of labile

OM into the soil, as previously indicated by CCF and FLF stocks. Leal et al. (2015b) reported higher CMI values (obtained by density fractionation) for a constructed soil covered for six years with four different grass species in comparison with a bare constructed soil. However, the authors stated that Hemarthria and Brizantha had similar CMI values, 565 and 612, respectively, and higher CMI values than the other treatments. These findings suggest that grasses, in this case Hemarthria, possibly require an adaptation stage to the conditions of the constructed soil, indicating that the performance of treatments must be monitored in the long term.

As expected, the CMI of natural soil was much higher (1,204) than the mean of grass-covered constructed soil (253) (Table 3). This difference probably resulted from the high degradation of the constructed soil and its recent revegetation. In addition, the non-disturbance of the natural soil together with the constant input of labile organic material, favor C accumulation in all OM fractions, improving the soil quality.

I_{LIF} and its relation with CMI

The I_{LIF} in the cover species treatments varied from 6,507 (T1) to 8,652 (T6), being lower than the I_{LIF} of bare constructed soil (8,905) and higher than that of natural soil (3,845) (Figure 2). The OM fluorescence results from the preferential excitation of organic compounds, whose concentration increases during humification (Milori et al., 2002). Therefore, the highest I_{LIF} of the bare constructed soil suggests the predominance of more humified organic compounds in OM, probably remnants of the natural soil, corroborating the data obtained by OM particle-size and density fractionation. In this treatment, the MAC/TOC (73 %) and HF/TOC ratios (87 %) were substantially higher than the other treatments, indicating the prevalence of non-labile OM. The lowest I_{LIF} of the natural soil was probably related to the constant supply of organic residues on soil surface and also the higher sand content in the surface layer of natural soil compared to constructed soil (Table 1). The sandier texture of the natural soil may have hampered OM accumulation in association with the mineral soil matrix (Santos et al., 2013).

The lower I_{LIF} in the cover species treatments than in bare soil indicate the entrance of labile OM in the soil and an improvement in the constructed soil quality after implantation of cover crops. On the other hand, compared to natural soil, the I_{LIF} of these treatments is still far superior, indicating that the OM of covered constructed soil consists predominantly of more humified and stabilized organic compounds, remnants of the natural soil and

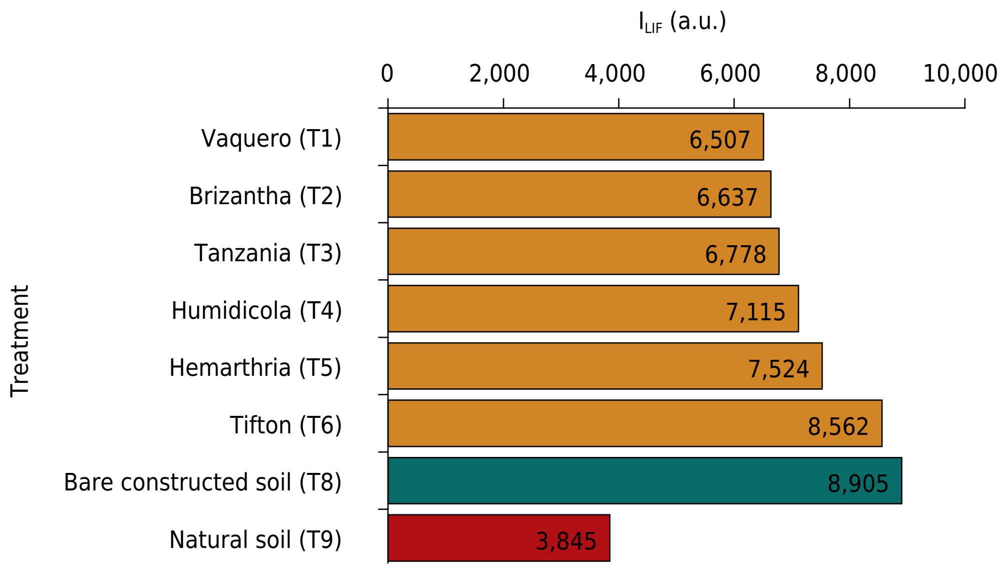

Figure 2. Laser-induced Fluorescence index (I_{LIF}) of organic matter in the 0.00-0.03 m layer of a constructed soil covered with different grass species, a bare constructed soil and a natural soil (unmined, with native vegetation).

associated with the soil mineral matrix. Among the cover species, the treatments Vaquero (T1), Brizantha (T2) and Tanzania (T3) resulted in lower I_{LIF}, indicating the potential of these species to add labile OM to the soil, corroborating data of CCF and FLF stocks.

The I_{LIF} was negatively correlated (r = -0.87; p <0.01) with CMI (Figure 3). The addition of labile organic compounds to the soil, as a result of cover crop cultivation, proportionally decreases the concentration of non-labile organic compounds in the soil, decreasing I_{LIF} and improving the soil quality, as evidenced by the concomitant increase in CMI. These data are consistent with those of Leal et al. (2015b). These authors reported a negative correlation (r = -0.82; p<0.05) between I_{LIF} and the humic acid (HA) stock in the 0.00-0.03 m layer of a soil constructed after coal mining (bare or grass-covered) and a natural soil. According to the authors, the increase in HA stocks proportionally decreased the C stocks in the most recalcitrant OM fractions, e.g., the humin fraction, increasing OM lability and consequently decreasing I_{LIF}.

Perspectives for recovery of the constructed soil quality

Long-term experiments are essential for monitoring the quality of constructed soils. Constructed in 2003, soil tillage/disturbance in the experimental area in Candiota occurred last in 2007, when the perennial grasses were sown in the beginning of the experiment, with no intention of soil tillage/disturbance in the future.

In the short and medium term, with the consolidation of the grasses along with the lower soil disturbance, a gradual improvement in the capacity and intensity soil properties is expected, until in the long term, the system reaches a new state of equilibrium (Reichert et al., 2016). In the same experimental area, Reis et al. (2014) observed that after about 33 months of grasses cultivation, especially Tanzania, decreased soil bulk density (BD), increased macroporosity, total porosity (TP) and aggregate diameter and decreased compaction level compared to the bare constructed soil. According to the authors, the better suitability of Tanzania for soil recovery may be related to the greater root density of this grass than of other grass species. However, future studies evaluating the root system of these species are needed, in view of the importance of the root system action for improvements of the soil physical structure (Sollins et al., 1996; Lima et al., 2012).

In a comparable experimental area adjacent to this study, Stumpf et al. (2014c) evaluated the effect of perennial grasses after 5, 41 and 78 months of cultivation on BD, TP and

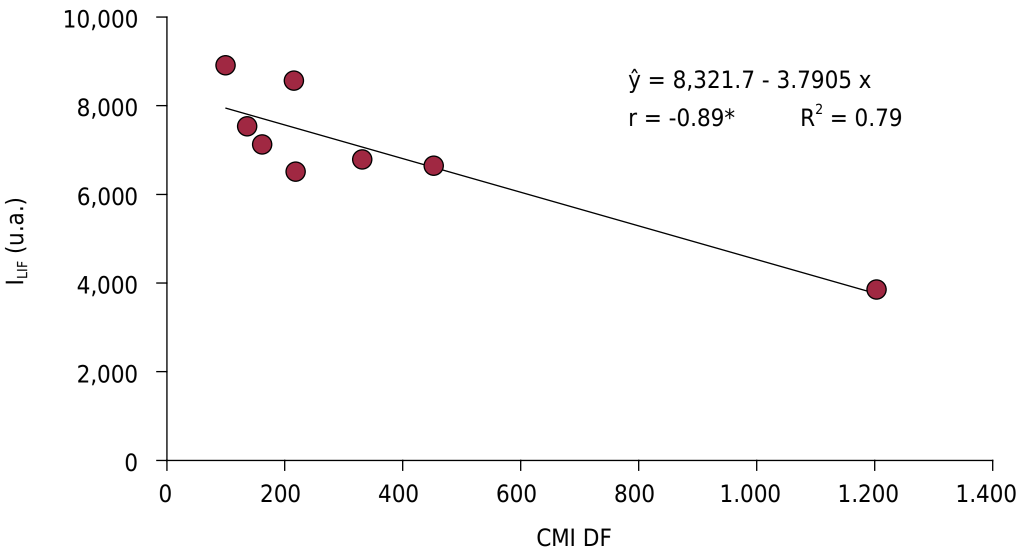

Figure 3. Relationship between the laser-induced fluorescence index (I_{LIF}) and carbon management index estimated from density fractions (CMI DF) of organic matter in the 0.00-0.03 m layer of a constructed soil covered with different grass species, a bare constructed soil and a natural soil (unmined, with native vegetation). *: significant, p<0.01.

macro and microporosity of a constructed soil. The authors observed improvements in these soil capacity properties throughout the revegetation period, indicating an ongoing process of soil quality recovery, i.e., that the equilibrium state of the system had not been reached, as expected.

A similar behavior can be expected for this experiment in the coming years. Under favorable conditions of soil conservation (plant adaptation, input of crop residues and non-disturbance of the soil), an improvement of the soil physical structure is expected, parallel to a greater C accumulation in both the labile and non-labile OM fractions. In this sense, the data of OM fractionation and characterization presented here serve as a baseline study for future monitoring of the quality of the constructed soil in Candiota.

CONCLUSIONS

After two years of cultivation, the grasses increased the stocks of total organic carbon and of carbon in organic matter fractions, except in the occluded light fraction.

The coarse and free light fractions were most sensitive in detecting the effect of different grass species on soil organic matter. The carbon accumulation in these fractions was favored by the cultivation of Brizantha and Tanzania.

In the covered constructed soil, regardless of the cover species, the intermediate carbon management and laser-induced fluorescence indices, between the bare constructed and natural soil, indicated a partial recovery of the soil quality.

The carbon stocks in the labile organic matter fractions and the carbon management index of constructed soil under Brizantha and Tanzania indicate a greater potential of these species in the initial recovery of the constructed soil. However, the effect of cover crops on the constructed soil must be monitored in the long term in order to confirm this potential.

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