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Soil attributes in coal mining areas under recovery with bracatinga (*Mimosa scabrella*)

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Significance and Impact of the Study: In coal mining areas under recovery with typically acid soils, the use of the current recovery strategies (revegetation mainly) has been efficient to increase the quality of soils, especially in the environmental restoration areas. Soil microbiological attributes such as microbial biomass nitrogen, microbial biomass carbon, microbial basal respiration and metabolic quotient (qCO_2) are dynamic and highly sensitive. These parameters have the potential to be adopted together with conventional attributes, such as floristic composition indices and species diversity indices, to evaluate the degree of any particular environmental recovery process being conducted at previously explored mining areas.

Keywords
bracatinga, coal mining, degraded areas, microbial basal respiration, microbiological attributes, monitoring, revegetation.

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Abstract

The coal reserves in the south of Brazil were intensely exploited at the time of great demand for such fuel. This resulted in changes in the environment, mainly in the chemical, physical and biological characteristics of the soil. Due to the potential to control erosive processes, increase soil quality and restore biological diversity, revegetation is a promising alternative to recover those impacted areas. In that respect, bracatinga is a pioneering tree species that easily grow in different environments and has being planted as vegetation cover in areas under recovery. Therefore, the objective of this work was to characterize the chemical features and to evaluate the soil microbiological attributes in areas degraded by coal mining and under recovery using bracatinga as cover plant. In the bracatinga canopy projection area, soil samples were collected in the environmental restoration areas that have been, at the time of collecting, under a regime of 2, 4, 6 and 12 years of restoration. In addition an area with natural occurrence of bracatinga was used as control. Microbial biomass nitrogen, microbial biomass carbon and microbial biomass respiration increase in average 281, 230 and 157% respectively, when the 12-year-old areas were compared to the 2-year-old-areas. Likewise, a decrease in qCO_2 in the order of 60% was observed for that same comparison. The 12-year-old areas reached the same values of qCO_2 found in the reference area. The data suggest an improvement in the microbiological attributes of the soil with the increase in recovery time for the studied areas.

Introduction

The coal deposits of economic importance in Brazil are located in the southern region of the country, comprising the states of Rio Grande do Sul, Santa Catarina and Paraná. The state of Santa Catarina has in its territory the second largest coal basin and it was intensely exploited both by underground and open pit mining (Soares *et al.*

2008). However, during the period of strong demand for fuel, the activity was developed without proper planning or observing the currently assumed recovery standards to maintain the quality of the environment surrounding the mined areas (Koppe and Costa 2008). The impact generated is associated with the inadequate disposition of tailings and sterile deposits, causing one of the region's most serious problems, known as acid mine drainage (AMD), as well as release of gases into the atmosphere, the occurrence of erosive processes and loss of extensive areas of native vegetation. Some of these impacts still persist in the region nowadays (Gaivizzo *et al.* 2002; Galatto *et al.* 2007; Koppe and Costa 2008; Campos *et al.* 2010). Because of the lack of planning to support the activity, the Southern Region of Santa Catarina was classified as the 14th national critical area for the purpose of pollution control and conservation of environmental quality.

For the recovery of those damaged areas, revegetation is a promising alternative, since the planting of a vegetation cover can ameliorate the natural erosive processes and improve the chemical, physical and microbiological attributes of the soil, which can facilitate, therefore, the on-site plant succession process (Sheoran *et al.* 2010). Therefore, the selection of plant species capable of developing in such environments is essential prior to the implantation of any restoration programme that rely on the vegetation as a component (Rocha-Nicoleite *et al.* 2013).

Bracatinga (*Mimosa scabrella* Benth.) is a pioneer, fast-growing species that is not very demanding regarding soil physical and chemical conditions, and therefore it is widely used in revegetation programmes in degraded environments (Carneiro *et al.* 2008). It is considered an early successional plant that facilitates plant succession due to the improvements it promotes in soil fertility, primarily by the deposition of nitrogen-rich litter, an element that is further transferred into plants symbiotically by diazotrophic micro-organisms (Callaway 1995). During its growth, bracatinga also stimulates microbial life in the soil, due to the large amount of nitrogen it incorporates and by the accumulation of organic matter (Poggiani *et al.* 1987). A study conducted by Stoffel *et al.* (2016) demonstrated the ability of young saplings to settle in soil containing coal mining tailings, with the addition that those saplings had the capacity to retain As, Cd, Cr, Cu and Zn in their shoots.

Although revegetation is part of a restoration practice, after plant establishment it is recommended to monitor the areas in order to evaluate if resilience is reached in the sites, and if success in the implementation is attainable. Currently, in the recovery areas of the Santa Catarina carboniferous basin, the use of environmental indicators is required which includes diversity attributes of fauna and flora involved in ecological processes (diversity, evenness, richness, etc.) capable of promoting the recovery of the ecosystem (Brasil 2016a). However, an important factor that influences resilience and the establishment of vegetation is the microbiota, which is often neglected in studies involving land reclamation.

Soil micro-organisms are the basis of ecological processes. They are responsible for supplying plant nutrients, regulating biogeochemical cycles of elements, including N and P, alter water infiltration, and ultimately are involved in the degradation of agrochemicals and heavy metals in the soil (Moreira and Siqueira 2006; Dominati *et al.* 2010; Aislabie and Deslippe 2013). Therefore, the role of the soil microbial community is vital for maintaining important relationships within the matrix that represents the components of the soil life.

Siqueira *et al.* (2008) emphasize that in the process of soil degradation, the biological activity is the first to be affected, and the last to be fully recovered during land reclamation. Environmental changes may negatively impact the growth and heterotrophic activity of soil micro-organisms (Odum 1985; Quadros *et al.* 2016). Thus, soil microbiological attributes, such as microbial biomass, basal respiration, metabolic quotient (qCO_2), activity of enzymes of soil , as well as microbial community structure, have been studied as indicators of changes in areas impacted by anthropic activities, thus serving as indicators of soil quality (Kaschuk *et al.* 2010; Araújo *et al.* 2013; Santos *et al.* 2013). In this way, this work aims to characterize the chemical attributes and to evaluate the microbiological attributes of the soil in areas degraded by coal mining under recovery with the species bracatinga.

Results and discussion

Soil chemical characterization

Currently there are different ages of revegetation programmes in the region of the Carboniferous basin of Santa Catarina representing a chronosequence of restoration, which provides an opportunity to study if there are variations in the physical, chemical and biological attributes over time. However, the absence of more than two areas with the same recovery time constitutes a bottleneck that limits us and can only infer how these processes are occurring. Meanwhile, this approach has great relevance because studies that evaluate the influence of revegetation on soil microbiological attributes are non-existent.

The chemical characterization of the soil is presented in Table 1.The data show that all areas have acid soils, in particular the area under 6 years of recovery, which presented the lowest pH. In relation to ΔpH, a predominance

of negative charges is observed, which favours the adsorption of ions with positive charges. The reference area (not mined) has a greater stock of C and N in the soil, as well as availability of K. In general, recovery areas recently planted have greater P availability due to the recovery processes that involves the application of organic materials (Table S1).

When trace elements were evaluated in the soil, they did not present values above the prevention values recommended by Companhia ambiental do estado de São Paulo (CETESB 2014). These prevention values refer to the concentration of trace elements, above which detrimental changes to soil or groundwater quality may occur. This value indicates the quality of a soil capable of sustaining its primary functions, protecting the ecological receptors and the quality of groundwater.

It is also important to highlight that the recovery processes employed in the region involve the application of organic materials that can increase soil total organic carbon (TOC) stocks, but this effect is temporary due to the heterotrophic activity of micro-organisms (Siqueira *et al.* 2008). In this context, revegetation assists in the supply and maintenance of organic material in the soil, both through rhizo- and/or litter deposition. Although it is a perennial species, bracatinga constantly loses its older leaves, depositing them in the soil. This process may deposit about 3500–4800 kg ha^{-1} $year^{-1}$ of litter depending on the plant age and density (Poggiani *et al.* 1987; Souza and Davide 2001). This characteristic is important both for promoting increases in organic matter contents and for improve the microbiological activity of the soil. Furthermore, the addition of litter can reduce the bioavailability of trace elements in the soil, since trace elements can be chelated by organic matter can be metabolized/complexed by soil micro-organisms (Bayer and Mielniczuk 2008; Ayangbenro and Babalola 2017). Nevertheless, it is possible to observe that even after the recovery procedures are implemented, the soils of the region are still highly acidity. The lowest pH values observed for the 6-year-old area can be therefore associated with the high nitrogen content of the organic material used for landscape restoration, which favours nitrification and, consequently, soil acidification.

Soil microbial attributes

Comparing the areas of coal mining revegetated with bracatinga, the 4-, 6- and 12-year-old areas show similar behaviour regarding the MBN, microbial biomass carbon (MBC) and microbial basal respiration (MBR) (Table 2), which do not differ statistically from one another. These areas had mean increments of 281, 230 and 157% for MBN, MBC and MBR in relation to the shortest recovery time area (2 years) respectively. However, they did not reach the values of MBN, MBC and MBR found in the soil with the natural occurrence of bracatinga, not impacted by mining.

In Table 2 it is also possible to observe that in the areas with shorter recovery times (2–6 years), the qCO_2, which allows to infer about the microbial activity of the

Table 1 Chemical analysis of soils from areas degraded by coal mining under different years of restoration in the Santa Catarina coal basin

	P	K	N_{Total}	TOC	pH	pH	
Areas+	mg kg^{-1}		g kg^{-1}		H_2O	KCl	ΔpH
2-year-old	23·17 ± 7·26*	65·53 ± 14·85	0·70 ± 0·15	5·03 ± 2·08	4·66 ± 0·05	3·79 ± 0·02	−0·87
4-year-old	74·55 ± 62·96	79·51 ± 40·04	1·80 ± 0·55	20·89 ± 9·06	4·53 ± 0·13	3·73 ± 0·09	−0·81
6-year-old	152·60 ± 57·94	78·38 ± 20·37	3·12 ± 1·14	49·59 ± 21·72	3·80 ± 0·21	3·33 ± 0·14	−0·47
12-year-old	8·14 ± 1·96	91·79 ± 49·81	1·55 ± 0·26	16·78 ± 3·47	4·91 ± 0·30	3·73 ± 0·15	−1·18
Reference	9·43 ± 2·49	126·06 ± 49·01	12·83 ± 3·31	212·09 ± 61·14	4·04 ± 0·38	3·53 ± 0·28	−0·51

	Zn	Cu	Mn	Cr	Pb	Cd	As
	mg kg^{-1}						
2-year-old	8·12 ± 1·66.	4·58 ± 0·95	166·37 ± 243·16	9·31 ± 2·58	20·74 ± 13·81	0·11 ± 0·06	2·40 ± 1·02
4-year-old	19·84 ± 5·66	7·79 ± 3·12	510·40 ± 463·33	5·60 ± 1·43	28·00 ± 18·28	0·12 ± 0·04	3·76 ± 1·90
6-year-old	22·43 ± 7·82	20·38 ± 8·28	37·56 ± 14·22	5·55 ± 0·85	25·96 ± 3·34	0·19 ± 0·05	5·27 ± 3·16
12-year-old	30·71 ± 7·44	7·86 ± 1·98	694·68 ± 374·71	5·14 ± 1·63	19·08 ± 12·89	0·12 ± 0·04	0·85 ± 0·23
Reference	14·29 ± 4·75	35·51 ± 8·70	128·01 ± 120·46	1·52 ± 3·94	9·68 ± 2·12	0·16 ± 0·02	13·53 ± 0·20
CETESB**	86	60	–	75	72	1·3	15

Δ pH obtained by the difference between the pH in KCl and H_2O.

TOC: total organic carbon.

*Data for mean ± standard deviation of the mean.

**Prevention values for trace elements recommended by CETESB (2014).

Table 2 Microbiological attributes of soils from areas degraded by coal mining under different years of restoration in the Santa Catarina coal basin

Areas	MBN	MBC	MBR	qCO_2	Fungi ($\times 10^4$) CFU ml per soil	Bacteria ($\times 10^5$)
2-year-old	8·53c*	81·25c	0·34c	4·18a	1·8ns†	4·8ns
4-year-old	28·63b	231·91b	0·92b	3·97a	3·0ns	8·0ns
6-year-old	24·20b	222·33b	0·83b	3·73a	2·1ns	6·6ns
12-year-old	44·69b	351·34b	0·87b	2·48b	3·9ns	12·7ns
Reference	323·06a	2252·93a	3·57a	1·59b	2·7ns	12·5ns

MBC: microbial biomass carbon (mg C kg per soil); MBR: microbial basal respiration (mg C-CO_2 kg per solo per h); qCO_2: metabolic quotient (mg C-CO_2 g per MBC per h); CFU: colony forming units.
*Data followed by the same letter in the column do not differ by Scott Knott's probability test at 5%.
†ns: *F*-test non-significant at 5% probability; MBN: microbial biomass nitrogen (mg N kg per soil).

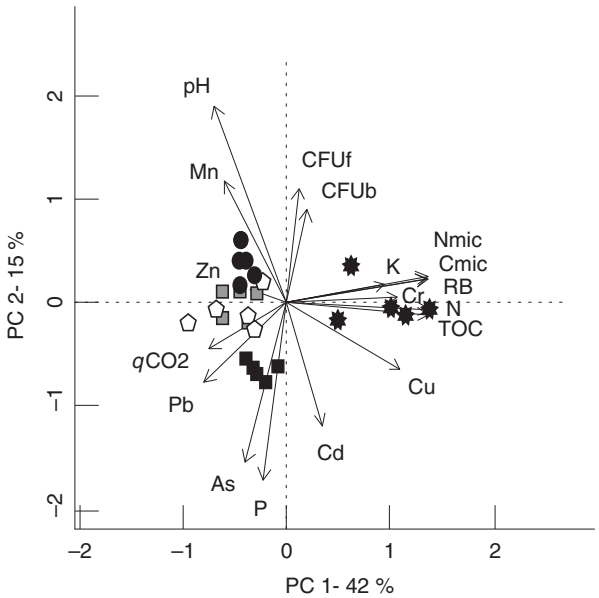

Figure 1 Principal component analysis of the chemical and microbiological attributes of soils degraded by coal mining in Santa Catarina coal basin. Sampling sites: (▨) 2-year-old, (⬠) 4-year-old, (■) 6-year-old, (●) 12-year-old of recovery and (✸) reference area.

soil, presented an average increase of 60% in relation to the area with 12 years of recovery, and 149% in relation to the reference area. However, the counts for bacteria and soil fungi were not significantly influenced by the treatments. The *q*mic values (TOC/MBC ratio), although not increasing proportionally with recovery time, ranged from 0·4 to 2·2% (data not shown).

With the results obtained, a multivariate exploratory analysis was performed through Principal Component Analysis (Fig. 1). The proportion of variances explained by the first two axes was 57%. It is possible to observe that the reference area presents an intense microbial activity (MBC, MBN, MBR, colony forming units of fungi (fCFU), colony forming units of bacteria (bCFU)) when compared to other areas. Furthermore, it can be verified that the areas with the shortest recovery time (2, 4 and 6 years) are associated with qCO_2, which according to Table 2 presented the lowest values for this variable.

From the analysis of the microbiological attributes of the soil, it was possible to verify different responses associated to the time of recovery. The carbon, nitrogen and basal respiration of the microbial biomass (MBC, MBN, MBR), although did not present values close to the bracatinga natural reference area, presented increases according to recovery time, indicating an improvement in the microbiological quality of the soil. However, the soil microbial enumeration is not a good indicator of recovery, which may be a limitation of the method that determines only cultivable organisms.

The microbial biomass is the most dynamic fraction of the organic carbon, being highly affected by soil changes (Santos *et al.* 2016). Thus, factors that negatively affect soil carbon supply also directly or indirectly affect microbial biomass (Santos *et al.* 2013). Therefore, strategies that promote the increment of this attribute in the soil are potential tools in the success of recovery programmes. In addition, the fraction of the microbial biomass of the organic matter, due to its constitution, represents an important reservoir of C, N and P, containing about 1–5% C, 2–5% N, and 2–20% P (Srivastava and Singh 1991). In the present work, the values of C and N in the microbial biomass, in proportion to the total, ranged from 0·5 to 2·2% and from 0·8 to 2·9% respectively.

Among the components of the microbial biomass, MBC is an example of attribute that is sensitive to changes in the soil and, therefore, has been one of the most evaluated microbiological attributes in studies of soils affected by trace elements (Renella *et al.* 2008). The MBC is highly influenced by the contributions of C and N in the soil, whose balance between the TOC and the mineral nitrogen should be sufficient to supply the growth of the soil microbiota, which are later made available as organic C and N through the death of microorganisms (Moreira and Siqueira 2006). Vegetation, through litter deposition and via constant and more easily decomposable deposition of organic material, can influence MBC and MBN, making these attributes highly related to the stages of recovery.

The results obtained in this work reinforce the studies supporting the MBC as a potential indicator of soil

degradation (Renella *et al.* 2008; Santos *et al.* 2013, 2016). Carneiro *et al.* (2008) verified that bauxite mining causes great impact on the soil, causing deficits of up to 99% in TOC, total nitrogen, MBC and soil enzymatic activity. However, revegetation of the mined areas promoted the recovery of the biochemical attributes of the soil, which were recovered faster (from 1 year) than the organic carbon and total nitrogen levels, which presented concentrations similar to those of the reference area, tending to stability starting from 18 years of rehabilitation or older. Quadros *et al.* (2016), in a study conducted to recover coal mining areas in the state of Rio Grande do Sul (Brazil), observed improvements in MBC after 19 years of the implementation of revegetation. Non-chronological studies by Santos *et al.* (2013) in areas contaminated with arsenic by gold mining, and Santos *et al.* (2016) in an area contaminated with trace elements used for the processing and industrialization of zinc, showed improvement in MBC after 11 and 8 years of the establishment of revegetation respectively. These results show that MBC may be an important microbiological attribute in the evaluation of degraded environments during land reclamation.

The MBR has little meaning when evaluated alone, since with the increase in microbial numbers it is expected an increase in respiration. However, the proportion between the microbial biomass and respiration becomes relevant, which characterizes the metabolic quotient of the soil (qCO_2). The value of qCO_2 makes it possible to infer about the efficiency of the microbial biomass in using the available carbon for biosynthesis, becoming a sensitive indicator to estimate the biological activity and the quality of the substrate (Saviozzi *et al.* 2002). The use of qCO_2 as an indicator to measure changes in soil quality is based in Odum (1985). According to that author, the increase in community respiration may be indicative of stressed environments, since repairing the damage caused by disturbances in the soil requires energy deviation from growth and reproduction for cell maintenance. Thus, such situations will lead the microbial biomass to direct more energy to cell maintenance, rather than growth, so that a proportion of biomass carbon will not be incorporated but lost as CO_2. With regard to qCO_2, it decreases as the recovery time increases. As a result, there is a greater incorporation than losses of C in the form of CO_2, indicating a decrease in stress favouring plant growth.

In the Carboniferous basin of Santa Catarina, the evaluation of areas under different years of restoration suggests that revegetation is successfully recovering the microbiological attributes of the soil. Micro-organisms, in turn, are both influenced and can influence the process of recovery by contributing to the improvement of the chemical and physical attributes of the soil. Micro-organisms can interact with the biotic environment of the soil, thus providing support for the ecological succession in areas under recovery.

Rocha-Nicoleite *et al.* (2013) mention that, following the implementation of forest restoration projects two categories of monitoring: implementation and sustainability, are required simultaneously, but with different objectives. However, the authors did not consider the microbiological attributes, which present fast responses with time, as shown in the present work, to provide more support in the inference about the progress of the recovery process of impaired areas, and can be evaluated jointly with other biological attributes.

Material and methods

Collecting sites and soil sampling

The recovery of the areas impacted by mining activities in the state of Santa Catarina began to be effectively implemented with the publication of the technical criteria for the recovery and rehabilitation of areas degraded by mining, put into action in 2006 (Brasil 2016b). The coal mining region of Santa Catarina has areas impacted by coal mining since the 1970s. However, official information on the implementation of the recovery processes is scattered and often incomplete.

Soils samples were collected in August 2013, in areas under 2, 4, 6 and 12 years of recovery (restoration programmes). The first two areas are located in the municipality of Lauro Muller and the following two in Treviso and Siderópolis, respectively, all located in the Santa Catarina state (SC) coal basin (Fig. S1). In those places soils are predominantly built (borrow soils), and therefore, created by anthropic means. In addition to the described areas, a reference area with natural occurrence of bracatinga was also selected, located in the municipality of Bom Jardim da Serra (SC). For the natural site, soil is classified as Histosols.

In all the studied areas, materials considered as contaminants (waste or sterile) were removed prior to the beginning of revegetation. Subsequently, materials (soil and substrates) were added for topographic conformation and to support the planting of a vegetation cover (tree and herbaceous species) (Table S1).

Soil samples were collected in the bracatinga canopy projection area in order to obtain a representative sample of the area under the influence of that species. In each site, five bracatinga individuals distanced each other by at least 50 m were selected and within a radius of 60 cm from the centre of each tree were obtained six subsamples spaced equidistantly in the projection of the canopy of

bracatinga. The six subsamples were joined to form a composite sample, so each tree corresponded to one repetition in each area under recovery, thus totalling 25 samples. A dutch type of stainless steel with a height of 120 cm whose compartment of soil collection allowed the sampling of the layer of 0–20 cm depth. The instrument used was flambeed prior to soil sampling. The organic residues from the soil surface were removed to grant accession to the soil surface. After being collected and homogenized in sterile bags, samples were fractionated for microbiological and chemical analyses. For the microbiological analyses, samples were refrigerated during transportation to the soil microbiology laboratory. In the laboratory, the samples destined to the microbiological analyses were kept under refrigeration (4°C), and those for the chemical characterization dried at room temperature.

Soil chemical characterization

A sample of the dry soils was sieved in a 2 mm mesh for the analysis of N, P and K, pH in water and pH in KCl. The ΔpH value was calculated from the difference between pH measured in KCl and the pH measured in water. N and C analysis were determined in an autoanalyzer (Shimadzu Corporation, Kyoto, Japan). For the quantification of the available levels of P and K in the soil, digestion was carried out according to Tedesco *et al.* (1995) and determined by the method proposed by Murphy and Riley (1962). The determination of pH in water and pH in KCl was performed according to EMBRAPA (1997). For the analysis of trace elements, the dry soils were passed through a 0·15 mm sieve, and samples digested following the method proposed by USEPA 3051a (USEPA 2013) Briefly, this method was performed in Teflon tubes (CEM, Mathews, NC) containing 0·5 g of soil sample and 5 ml of concentrated HNO_3 using a Cem Mars 5 microwave (CEM Corporation, Matthews, NC) at a pressure of 0·76 MPa for 10 min. After cooling, the contents were filtered and diluted. Analytical quality was verified using standard reference materials NIST-SRM 2710a. All determination was carried out in triplicates. The quantification of Zn, Cu, Mn, As, Pb, Cd and Cr was determined by atomic absorption spectrometry using the AAnalist 800 equipment (Perkin Elmer, Ueberlingen, Germany).

Enumeration of soil bacteria and fungi

Sample of 10 g of soil was transferred to a 250 ml Erlenmeyer flask containing 90 ml of saline (0·85%), agitated at 120 rev min^{-1} for 20 min. Serial dilutions of 10^{-2}–10^{-5} and 10^{-1}–10^{-3} were performed for counting colony forming units of bacteria bCFU and fungi fCFU respectively. Dilutions were plated on the surface of specific culture media in triplicates and incubated at 28°C for 3 days. For bacterial counts, tryptone soy agar medium was used (g l^{-1}): 15 pancreatic casein digestion; 5 sodium chloride; 5 papaic digest of soybean meal; 15 agar; 0·04 of the fungicide pentachloronitrobenzene; pH 7·3. Dichloran Agar of Bengal Chloramphenicol medium was used for counting of fungi (g l^{-1}): 10 glucose; 5 soy peptone; 1 KH_2PO_4; 0·5 $MgSO_4.7H_2O$; 0·03 Rose Bengal; 0·002 Dichloran; 0·1 Chloramphenicol; 15 agar; 0·03 streptomycin and 0·05 tetracycline; pH 5·7. Both media were sterilized at 121°C for 20 min, with the exception of the streptomycin and tetracycline antibiotic solutions, which were filter sterilized (0·22 μm) and posteriorly added to the medium.

Nitrogen and carbon microbial biomass

The determination of C and N of the microbial biomass was done by the fumigation-extraction method (Vance *et al.* 1987). After fumigation, C and N were extracted with a solution of 0·5 mol l^{-1} K_2SO_4. Then, extracts were read in a C and N autoanalyzer (Shimadzu Corporation), using a correction factor of 0·40 and 0·54 for C and N respectively (Kaschuk *et al.* 2010). Non-fumigated samples were subjected to the same procedure, except the exposure to chloroform.

Microbial basal respiration

The basal respiration of a soil consists of the value obtained from the amount of C-CO_2 emanated from the samples during incubation, reflecting the microbial activity. The CO_2 produced by the micro-organisms during respiration is captured in a solution of NaOH. The method proposed by Jenkinson and Powlson (1976), with adaptations, was used to incubate the soil in hermetically sealed flasks containing 10 ml NaOH 0·5 mol l^{-1}. The vials were incubated for a period of 7 days in a BOD incubator at 25°C. The respiration rate was determined from the titration with 0·5 mol l^{-1} HCl, adding 1 ml of 10% (w/v) $BaCl_2$ and two drops of phenolphthalein 1% (w/v) to the remaining NaOH. The controls were prepared using non-soil flasks, and analysis carried as described above. The metabolic quotient (qCO_2) was obtained from the ratio between basal respiration per unit of carbon of the soil microbial biomass (Anderson and Domsch 1993).

Statistical analysis

The data for the microbiological attributes were transformed into log (x), submitted to an analysis of variance

and the Scott Knott's means separation test (5%) employed using the statistical program ASSISTAT ver. 7·7 (Universidade Federal de Campina Grande, Campina Grande, Brasil). Principal component analysis was performed as an exploratory multivariate analysis technique, for which both variables were standardized and analysis performed using the R software (R Core Team 2011).

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Figure S1 Location of the sampling sites in the Santa Catarina coal basin. (A2) 2-year-old, (A4) 4-year-old, (A6) 6-year-old, (A12) 12-year-old of recovery and (RA) reference area.

Table S1 Characteristics of the areas in different stages of recovery in the Carboniferous region of Santa Catarina.