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Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol☆

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Summary

The influence of charcoal and smoke condensates (pyroligneous acid, PA) on microbial activity in a highly weathered Amazonian upland soil was assessed via measurements of basal respiration (BR), substrate-induced respiration (SIR), and exponential population increase after substrate addition. PA extracts are commonly used for fertilizer or as pest control in Brazil, where phosphorus (P) availability and nitrogen (N) leaching are among the most severe limitations for agriculture. Microbes play an important role in nutrient cycling and solubilizing of phosphate. BR, microbial biomass, population growth and the microbe's efficiency (expressed by the metabolic quotient) increased linearly and significantly with increasing charcoal concentrations (50, 100 and 150 g kg^{-1} soil). Application of PA caused a sharp increase in all parameters. We suppose that the condensates from smoke contain easily degradable substances and only small amounts of inhibitory agents, which could be utilized by the microbes for their metabolism.

Introduction

In many parts of Brazil charcoal or carbonized biomass (rice hulls) are used in agriculture (Steiner et al., 2004b) and distillation of the pyroligneous acid (PA) fraction is often part of the

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charcoal-manufacturing processes. This technique comes from Japan where PA has been used for centuries to increase crop productivity and quality and to combat diseases and pests in agriculture (Zanetti et al., 2003). So far, not much is known about the chemical composition of the product, which consists of more than 200 chemical compounds (Encarnacao, 2001; Glass, 2001). In Brazil, a growing number of organic farmers have begun the production of PA (Glass, 2001).

To produce PA, gases from the charcoal kiln are channelled in such a way as to allow condensation of the vapour. The condensed PA consists mainly of water-soluble chemical species such as acids, alcohols, aldehydes, ketones and sugars. The high concentration of acids (as much as 25% by weight) gives PA a low pH ($pH < 3$) and in combination with the formaldehyde present, gives PA antifungal and other pest-control properties (Diebold, 1999). In spite of the lack of significant research, this by-product of charcoal production is used to improve soil physical, chemical and biological properties. Compost production is accelerated if applied to organic materials with diminished gaseous N losses (Encarnacao, 2001). It is supposed to favour beneficial microorganisms such as actinomycetes and mycorriza (Miyasaka et al., cited in Zanetti et al., 2003). In contrast, the extract is also used as soil disinfectant (Doran, 1932), as fungicide (Numata et al., 1994) and to control nematodes (Cuadra et al., 2000).

It is a common practice to apply the extract together with charcoal residues. Carbonized materials are formally authorized for use as soil amendment material in Japan (Okimori et al., 2003). The exceptionally fertile manmade Terra Preta soils in the Brazilian Amazon contain large amounts of charcoal and thus provided the incentive to study charcoal as a soil amendment. We described slash and char as an alternative to slash and burn (Lehmann et al., 2002; Steiner et al., 2004b), whereby a big proportion of the carbon in woody biomass is converted into charcoal instead of carbon dioxide. Charcoal amendments to soil were reported to improve soil fertility, plant nutrient uptake and crop production (Steiner et al., 2007), and reduce leaching of applied mineral N fertilizer (Lehmann et al., 2003). DeLuca et al. (2006) found increased net nitrification in charcoal amended soil as a result of greater activity of the nitrifying community. The soil microbial community seems to play a major role in Terra Preta formation and in sustaining soil fertility. Woods and McCann (1999) believe that the main human contribution toward the darkening of Terra Preta was not through primary deposition, but rather was the indirect result of chemical changes that stimulated soil biota activity.

In order to evaluate the applicability of slash and char and to gather further information about the mechanisms of Terra Preta formation and sustainability, we assessed the effect of charcoal and PA on the soil microbial community. Substrate-induced respiration (SIR) and microbial biomass have been shown to be appropriate measurements to assess the effects of management practices (Stenström et al., 1998; Steiner et al., 2004a) or toxic agents (Beck and Bengel, 1992) on the soil microbial community. Since microbial growth is often limited by the availability of nutrients, we hypothesize that charcoal amendments may reduce the growth of the microbial population, due to the potential nutrient-absorbing capacity. As PA is used to control pests and diseases we hypothesize that toxic effects might reduce the microbial population size, activity and population growth. Due to the charcoal's recalcitrance (Skjemstad, 2001), we do not expect significant alterations in basal respiration (BR).

Materials and methods

Study location

This study was conducted in central Amazonia (Brazil) at the Embrapa-Amazônia Ocidental (Empresa Brasileira de Pesquisa Agropecuaria) station, 30 km north of Manaus (3°8′S, 59°52′W, 40–50 m a.s.l.). The mean annual temperature is 25.8 °C (1987–1997), with an average relative humidity of 85% (Correia and Lieberei, 1998).

Experimental design (Study A)

Fifty kilograms of topsoil (0–0.1 m) was taken from an experimental bare soil area and sieved (<4 mm, Table 1 for chemical characteristics). The soil was fine-textured with high clay contents, strongly aggregated and had medium contents of organic C, low pH values, low CEC ($1.6\,cmol_c\,kg^{-1}$) and low base saturation (11.2%).

In previous field studies, chicken manure-amended soil showed durable high microbial activity and population-growth capacity of the microbial community in a 2-year period (Steiner et al., 2004a); chicken manure was mixed with the soil to ensure a high microbial activity. The manure was applied at a rate of $65\,Mg\,ha^{-1}$ ($65\,g\,kg^{-1}$) in the first 10 cm of soil. The organic matter-amended soil was stored in a box for 10 weeks in the dark at a humidity of 28%.

Table 1. Characteristics of the studied samples before and after addition of chicken manure (CM).

	N ($g\,kg^{-1}$)	P ($g\,kg^{-1}$)	K ($g\,kg^{-1}$)	Ca ($g\,kg^{-1}$)	Mg ($g\,kg^{-1}$)	C ($g\,kg^{-1}$)	pH H_2O	pH KCl
Original soil	1.59	0.007	0.020	0.009	0.007	17.96	3.9	3.8
Soil+CM	2.21	0.94	3.64	6.71	0.83	30.92	6.7	6.8
Charcoal	5.39	0.026	0.23	0.82	0.17	780	—	—

Table 2. Treatments and factors – Study A and B assessed the influence of charcoal on the microbial population.

Treatment	Study A Humidified[a] charcoal amendment ($g\,kg^{-1}$ soil DW)	Study B Kaolin/charcoal amendment ($g\,kg^{-1}$ soil DW) (ml H_2O)	Study C (2^4 factorial design) amendment ($g\,ml^{-1}\,kg^{-1}$ soil DW)
C (control)	0	150/0+3	(a) 50 g charcoal/kaolin (b) 12.5 ml PA/without (c) 50 ml H_2O/without (d) 6 g glucose/without (after basal respiration)
CI	50	100/50+3	
CII	100	50/100+3	
CIII (Charcoal III)	150	0/150+3	
CIII+H_2O	150+5 ml H_2O		

In Study A humidified charcoal (soil's humidity) was used and Study B was based on equal sample weight with kaolin substitution. Study C used a factorial design to investigate the influence of pyroligneous acid (PA) on soil respiration.
[a]Adjusted to soil moisture content; Study A CI, CII, CIII = charcoal treatments with rising charcoal contents; Study B kaolin/charcoal DW (e.g. 150/0 = 150 g kaolin and 0 g charcoal kg^{-1} soil); Study C factorial design 4 factors and 2 levels: (a) charcoal or kaolin; factor (b) PA with or without; factor (c) soil moisture; factor (d) glucose.

To assess effects of charcoal, portions of 40 g of soil (dry weight) were amended with 0 (treatment C), 2 (treatment CI), 4 (treatment CII), or 6 g (treatment CIII) charcoal powder (0, 50, 100, 150 $g\,kg^{-1}$, respectively,) prior to measurement (Table 2). Charcoal derived from secondary forest wood, was bought from a local distributor. It was manually crushed and milled to powder (see Table 1 for nutrient contents). The humidity of the charcoal powder was equalized to that of the soil. A fifth treatment got 6 g humid charcoal powder and additional 5 ml of H_2O to study the influence of humidity.

Charcoal with kaolin substitution (Study B)

The different charcoal additions caused different weights of the samples (40–46 g DW). Therefore, a second measurement was done substituting charcoal with kaolin. The dry charcoal and kaolin powder was considered to be relatively free from microbes. In the second measurement all samples were amended with 3 ml H_2O and charcoal or kaolin was applied dry (Table 2). Each treatment was measured in triplicate.

Application of PA (Study C)

The effect of PA on microbial respiration was tested in a factorial design (Table 2). A volume of 0.5 ml of the commercial product "biopirol" (Biocarbo, Itabirito, MG, Brazil) mixed with 2 g charcoal or kaolin was added per 40 g soil. Two millilters H_2O accounted for the "humidity" factor.

Microbial respiration and biomass

The respiration of soil samples was determined by hourly measurement of carbon dioxide (CO_2) production for each sample over a period of 65 h in a continuous-flow system at a constant flow rate of 300 ml ambient air per minute. The ECT-Soil Respiration Device (ECT Oekotoxikologie GmbH, Germany) based on infra-red gas analysis (IRGA) was used, according to the procedure described by Förster and Farias (2000) and Förster et al. (2006). The SIR method is a physiological method for the measurement of the soil microbial biomass. When easily degradable substrates, such as glucose, are added to a soil, an immediate increase of the respiration rate is observed, the size of which is assumed to be proportional to the size of the microbial biomass (Stenström et al., 1998). The BR is measured without the addition of a substrate, while the SIR is measured shortly after the substrate (240 mg glucose) addition. Glucose (240 mg = 6 g kg^{-1}) was applied after 10 h BR measurement.

Microbial respiration was calculated according to

$$\text{respiration [nL } CO_2 \text{ min}^{-1}\text{ g}^{-1}\text{ soil]} = (CF)/S, \quad (1)$$

where C is the CO_2 concentration (ppm), F the flow rate through cuvette (mL $min^{-1)}$, S the soil net dry weight (g) without including the charcoal additions. The dry charcoal powder was assumed to be relatively poor in microbial life.

Microbial biomass was calculated according to (Anderson and Domsch, 1978):

$$\text{microbial biomass } (C_{mic})[\mu g\ C_{mic}\ g^{-1}\text{ soil}] = (R \times 40.04) + 0.37, \quad (2)$$

where R is the respiration ($\mu L\ CO_2\ g^{-1}\ h^{-1}$).

The factor 40 was contrived for soils in temperate regions but used for tropical soils by Förster and Farias (2000).

The specific respiration increment was quantified as the slope of the exponential respiration increase after substrate addition when the respiration rate is plotted on a scale against time. This slope was described by

$$N = N_0 e^{kt}, \quad (3)$$

where N_0 is the initial concentration of microorganisms, k is the specific growth rate, and t is time.

The following parameters served as indicators of soil quality, organic matter turnover (Figure 1) and nutrient availability: BR, OM turn over, SIR, velocity of population increase (k) after substrate addition (nutrient availability and soil quality), microbial efficiency expressed as the metabolic quotient as CO_2 production per microbial biomass unit (CO_2-C h^{-1} C_{mic}^{-1}).

Statistical analyses

Treatment effects were analysed using analysis of variance (ANOVA). Homogeneous subsets were separated by the least significant difference (LSD) test. Levine's test of equality of error variances was applied. The influence of PA, glucose, charcoal and humidity were assessed using a complete 2^4 factorial design (two levels, four factors). The effects of the factors and their interactions were estimated by applying Yates' algorithm according to Morgan (1991). The results of the algorithm were used to calculate the sum of squares for an ANOVA test. The 3- and 4-factor interactions were assumed to be negligible and were combined to yield a measure of residual error. Statistical analyses and plots were performed using SPSS 12.0 and SigmaPlot (SPSS Inc.).

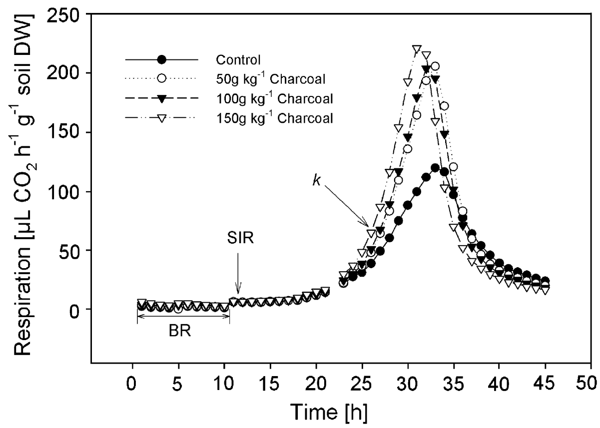

Figure 1. Respiration of charcoal amended soils (2, 4 or 6 g per 40 g soil dry weight) in comparison with a control (0 g charcoal). Basal respiration (BR), substrate induced respiration (SIR), glucose was added after 11 h of BR measurement) and the exponential respiration increase caused by an exponential population growth ($N = N_0 e^{kt}$). The area below the curve (from h 11–45) shows the amount of released CO_2 (metabolized glucose). Error bars are not shown to improve the clearness of the figure. The measured parameters and results are shown in more detail in Figure 2.

Results

Effects of soil charcoal amendments

Charcoal significantly increased all microbial parameters measured in both trials (constant weight with kaolin substitution or not). The respiration curves of the two studies were very similar; therefore, we describe the respiration curves with kaolin substitution in more detail, referring only to charcoal alone when significant differences occur.

Charcoal gradually decreased the time to reach the respiration maximum after glucose addition from 23 to 21 h from treatment C to CIII (0–150 g charcoal kg^{-1} soil, respectively). In the following 35 h after substrate addition the CO_2 release was 38.5% of the carbon applied as glucose in CIII and only 29.6% without charcoal (Figure 1).

The BR increased linearly and significantly ($P < 0.001$) along with increasing charcoal concentration (50, 100 and 150 g kg^{-1}, Figure 2a). The addition of water caused a further significant

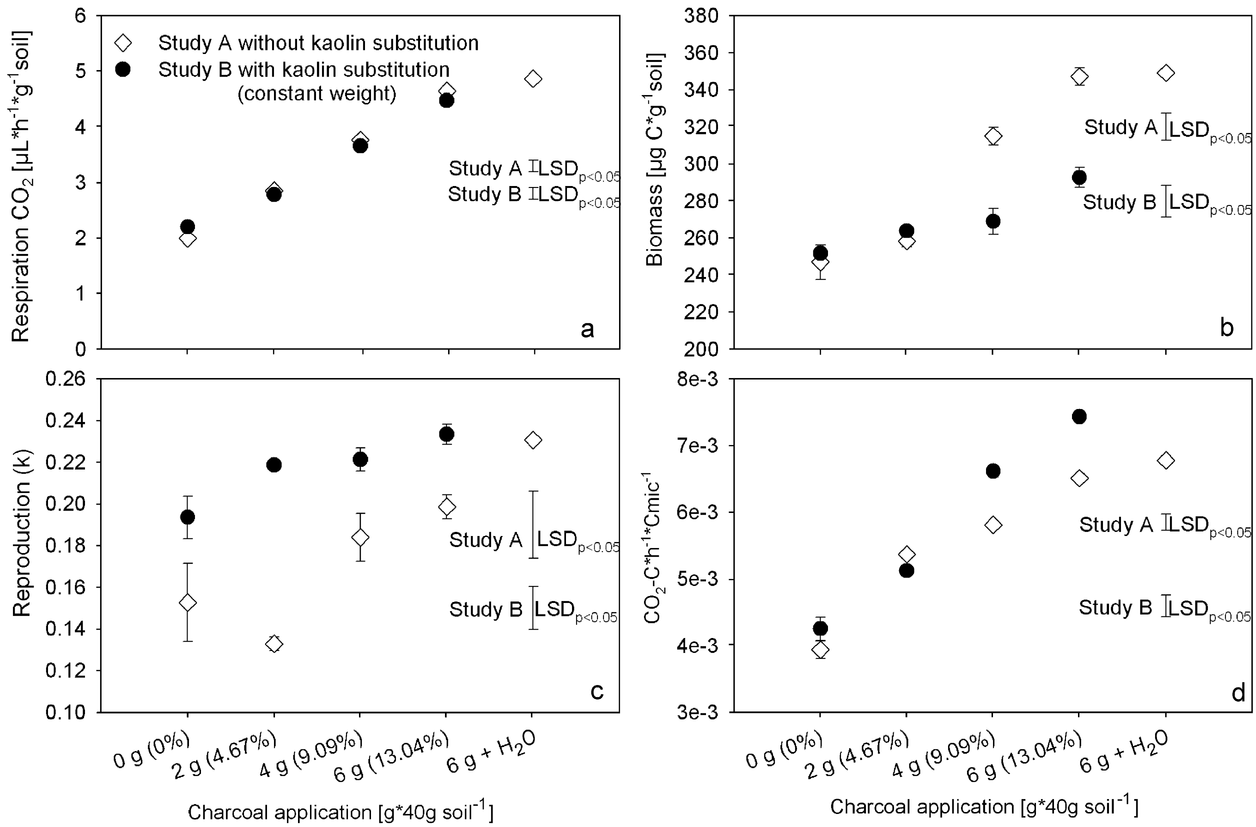

Figure 2. (a) basal respiration, (b) microbial biomass, (c) reproduction potential (k, $N = N_0e^{kt}$), and (d) CO_2 production per microbial C (metabolic quotient) with increasing soil charcoal concentration. Study B was performed with constant sample weight by kaolin substitution; means and standard errors ($n = 3$). In some cases the error bars are smaller than the symbols.

increase in BR, presumably because optimum water and oxygen supply is assumed at relative humidity between 40% and 60% of the maximum water-holding capacity of the soil. In both studies, the microbial population (Figure 2b) and population growth (Figure 2c) increased significantly due to charcoal application. Increasing charcoal concentration caused a steep linear increase in microbial efficiency ($P<0.001$), expressed as the metabolic quotient (Figure 2d). Soil moisture also increased the metabolic quotient significantly ($P<0.05$).

Effects of PA

PA and humidity increased the BR, microbial biomass and the population growth rate (k) significantly ($P<0.01$). Considerable population growth occurred during measurement of BR (Figure 3). It is remarkable that microbes utilized PA for their metabolism. SIR was also strongly influenced by the PA. After glucose addition, microbial population growth was much faster if the soil contained PA, because population growth was already initiated (Figure 3). The influence of charcoal (data not shown) was small in comparison with that of PA.

Discussion

Effect of charcoal with or without kaolin substitution

Because of the almost identical data obtained for BR in the 2 studies (Figure 2a), we infer that the differences observed in microbial biomass, population growth potential and metabolic quotient are due to experimental conditions and not sample handling. Charcoal by itself (study A) will have a higher percentage of the total sample after amendment (w/w); therefore it may contribute to a greater affect on microbes as the effect seems mediated by surface area. The microbial biomass increased significantly ($P<0.05$) with increasing charcoal application although to a smaller extent with kaolin substitution (Study B, Figure 2b). The difference could be due to the different methods of

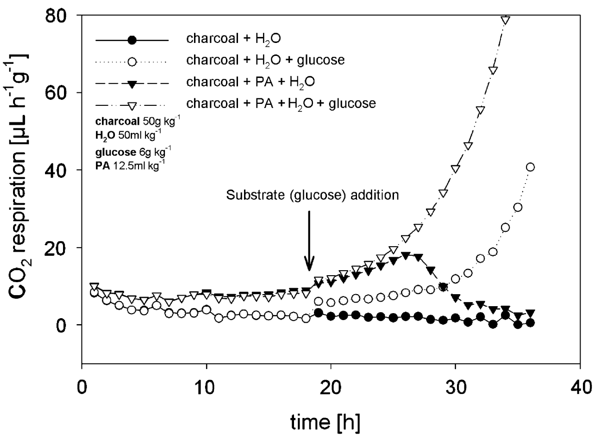

Figure 3. The influence of pyroligneous acid (PA) and glucose (added after 18 h) on soil respiration. The amendments were added to 40 g soil DW. Only selected treatments are plotted. Remarkable is the small deviation between the same treatments before glucose addition. PA without glucose addition (▼) caused an exponential growth ($k = 0.07$, $R^2 = 0.97$) with an earlier peak.

water application. In Study A water was applied proportionally with charcoal, whereas water was applied at a constant rate in Study B. Higher soil-moisture content increases the availability of dissolved nutrients. Additional water application increased the population growth potential significantly ($P = 0.052$) and was responsible for the faster population growth in study B. Therefore, the higher charcoal application rates (100 and 150 g kg^{-1}) show the biggest differences. We believe that mixing charcoal into the soil and wetting caused a microbial population increase or an activation of dormant microbes in the soil. The fresh charcoal was considered to be relatively free from microbes. The longer BR measurement in study A (17 h) in comparison with study B (11 h) and the even earlier wetting of charcoal in study A might have caused the sharper increase in microbial biomass due to charcoal application in study A. The microbial biomass can represent a large pool of nutrients especially on less fertile sites (Giardina et al., 2000). Microorganisms can act as sinks, during immobilization and sources, during mineralization of labile nutrients (Garcia-Oliva et al., 1998; Stenström et al., 1998). Immobilization may prevent N losses into subsurface horizons or gaseous losses into the atmosphere (Bengtsson et al., 2003) and, therefore, may be important as a nutrient-retention mechanism in those soils highly affected by leaching and volatilization. It is questionable how far charcoal-C can be utilized by microbes and thus favouring N immobilization. Furthermore, P, which is the primary limiting nutrient to plant production in highly weathered soils of the humid tropics, could become available through fine root endomycorrhizal associations (Garcia-Montiel et al., 2000) and through heterotrophic phosphate solubilizing microorganisms, which are supposed to be stimulated by soil charcoal additions (Kimura and Nishio, 1989). The recalcitrant nature of charcoal and low-nutrient contents therefore, makes charcoal itself unlikely to be a balanced fertilizer, but is important to support a healthy biological activity.

Charcoal amendments significantly increased the reproduction rate of microbial population after substrate addition in a field trial, even with charcoal additions of only 12 g kg^{-1} soil (0.5/40 g soil), whether fertilized or not (Steiner et al., 2004a). The same was observed in Terra Preta soils, although the BR and SIR remained as low as the control. Responsible for the increased BR in charcoal amended soil might be the easily decomposable fraction (bio-oils, or PA) of the charcoal, if charcoal is applied in un-weathered condition. A much longer in-field incubation time for charcoal might be required to observe a BR as low as on control soil. As charcoal is metabolized, leaving the recalcitrant fraction which is supposed to be a preferred habitat for microbes (Ogawa, 1994) and that can support a more active microbial community (Pietikainen et al., 2000). This may cause the effects (low BR and high population growth potential) observed on Terra Preta.

Effects of PA

A list of substances found in smoke was given by Fischer and Bienkowski (1999). All these compounds can be consumed by strains of prototrophic bacteria occurring in the soil (Focht, 1999). Typically, condensate PA is high in low-molecular weight acids (formic and acetic), alcohols (methanol) and aldehydes (formaldehyde and acetaldehyde) (Diebold, 1999; Focht, 1999). Some of the largest constituents are acetic acid (0.5–12.0% by weight of total condensate), formic acid (0.3–9.1%), methanol (0.4–2.4%), formaldehyde (0.1–3.3%), acetaldehyde (0.1–8.5%), and hydroxyacetaldehyde (0.9–13.0%) (Diebold, 1999). If the concentration of PA applied is high, then formaldehyde and acids can serve as biocides (Doran, 1932). However, at low concentrations these alcohols, acids, and aldehydes serve as carbon and energy substrates for soil microorganisms.

Our estimates of the amount of available carbon present in the PA addition based on typical composition described by (Diebold, 1999) indicate a range between 31 and 207 mg C (Average 119 mg C). This total carbon is derived from 21 different compounds (including acids, aldehydes, alcohols, ketones and anhydrous sugars) and hence would be less readily utilized than glucose. In contrast, glucose addition (96 mg C) provides a substrate that microbial metabolism can use directly, thus providing rapid population growth. Antal and Grønli (2003) wrote that the tar vapour from charcoal production is composed of a complex reactive mixture of organic compounds including vapour-phase sugars, and anhydro-sugars and their oligomers, fragments of sugars, and lignin moieties. These are highly unstable at elevated temperatures and rapidly decompose on the surface of charcoal, producing secondary charcoal and a gas composed primarily of water, carbon dioxide, methane, hydrogen, and carbon monoxide. In this study, the temperature was 21 °C and the exponential increase of CO_2 production (Figure 3) demonstrated a microbial mediated decay of PA. Fischer and Bienkowski (1999) and Uvarov (2000) found that contaminated soil had a clearly enhanced respiratory metabolism after long-term exposure to smoke emissions from charcoal production in Poland.

Conclusion

Slash and burn is causing considerable environmental damage, including soil degradation (Tiessen et al., 1994), emissions of greenhouse gases (Fearnside, 2000) and on a regional scale severe air pollution. The carbonization of biomass for soil amelioration purposes would reduce CO_2 emissions and even establish a carbon sink if re-growing resources are used. Charcoal can be produced in the wet season, when burning is not possible. Controlled year-round charcoal production would distribute emissions around the year and reduce the high aerosol emissions during the dry season.

The production of PA from smoke decreases the environmental impact further (Encarnacao, 2001).

Phosphorus availability and nitrogen leaching are among the most severe limitations for agriculture in the Amazon Basin and the soil microbial community plays an important role in nutrient cycling and availability. Charcoal and PA clearly alters the soil microial activity. It remains a challenge to discern the charcoal's role in Terra Preta formation and sustainability.

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