

Microbial respiration of novel biomixtures used for biopurifications system

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ABSTRACT

To overcome the problem of on farm point sources of pollution stemming from improper handling, spillages and leakages during filling and cleaning of spraying equipments, environmental friendly and low cost technology filter systems are under development. Biochar and digestate mixtures with two types of soil (sandy and silt loam) had been used as a biofilter test material for a respiration study instead of conventional soil, peat and straw mixtures. The results show that digestate is an easily available C-source leading to highest release of CO₂-C compared to all other biomixtures used in the present experiment. It was found that with the addition of even a small amount (1 % W/W) of biochar there is a profound suppression in the CO₂-C release.

Keywords: Biochar, biomixture, degradation, digestate, point source of pollution, respiration

Unsustainable management of hydrophobic organic contaminantssuch as pesticides, PAH compounds like naphthalene, pyrene, benzopyrene etc. can cause high concentrations in soils, ground and surface waters. This is perceived as a major environmental issue in Europe (Kolpin *et al.*, 1996; Kolpin *et al.*, 1998; Kreuger, 1998). In general, sources of pollution are categorized into diffuse and point sources. Diffuse contamination *via* leaching, runoff, surface drainage, and drift usually contributes only to a smaller part of pollution of ground and surface water. Many observations at the catchment scale have demonstrated that 40 to 90% of surface water contamination by pollutants is due to point source pollution (Mason *et al.*, 1999; Carter, 2000; Ramwell *et al.*, 2004), which is caused by spillages during storage and filling, as well as imprudent management of remnants, stemming from leftover spray solution and sprayer washing water. Point source pollution can be avoided or significantly reduced by applying best management practices in the area of daily routines (*e.g.* sprayer washing). To further decrease of the standards of European Drinking Water Directive (DWD), a widespread implementation of cost-effective technologies is required to reduce point source pollution on farmsteads. A possible approach to safely dispose remnants of crop protection products is the use of biobeds or biofilters (biopurification systems).

Our hypotheses are:

- a) When digestate and chars will be used for as a novel filter material they will be able to sorb and degrade studied pollutants effectively that may lead to reduced leaching and less chances of ground water pollution.
- b) Chars being highly microporous in structure and

having high surface areas might act as “super sorbents”. On the other hand, digestate with more easily degradable C and lower C/N ratio will lead to enhanced microbial growth and enzymatic activities that may relate to degradation of pollutants in later stages.

We will test these hypotheses in our study with different biomixtures obtained by mixing of soils with different organic residues *i.e.* digestate and chars.

MATERIALS AND METHODS

Soils and organic amendment used in the incubation experiment

Two contrasting soil types (Sandy soil, a Gelid Cambisol, taken from the Kaldenkirchen field location and another Silt loam soil, an orthic Luvisol, taken from Merzenhausen field site, Germany) (Koestel *et al.*, 2009; Garre *et al.*, 2011) were selected for sample or matrix preparation for further process understanding and incubation study, whereby these soils were mixed with three different organic amendments such as low temperature (400°C) and high temperature (800°C) char and digestate. Both chars were obtained from slow pyrolysis processes using woodchips as feedstock and the digestate added which was obtained from anaerobic digestion process using chicken manure, beef waste, and maize silage as a feedstock.

Preparation of soils with organic amendments

Triplicates of field-moist soil samples were sieved (2 mm), and kept at 5 ± 2 °C until further analysis. The preparation of pot experiment was carried out with 3 kg (dry weight equivalent) soil in 12 L plastic pots at 20 ± 5 °C. Soil moisture content was determined separately, and de-ionized water was added to adjust the soil to 20 % of the soil maximum water holding capacity (WHC_{max}). Subsequently, the different organic residues

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or amendments were thoroughly mixed in suitable ratios at this moisture stage. After rewetting, the soil was stored in the dark at 20°C for 3-4 days to re-establish soil humidity equilibrium and to reactivate the soil microflora. The final moisture content was adjusted to 50 % of WHC_{max} by adding de-ionized water. The pots were covered by a polyethylene cover, to allow for a certain gas exchange, but to retard excessive evaporation and the soil moisture was gravimetrically adjusted with de-ionized water for each individual pot once a week. The experiment comprised of 14 different treatments for each of both soils: one control (bare soil without any amendments) and 13 different application ratios of organic residues or amendments. Mixture of soils with organic amendments kept in the pots for 60 days. The concentrations of char applied were 1, 2.5, and 5 % and for digestate 5, 15, and 30 % respectively. Additionally, a mixture between char and digestate with a concentration of 1 % (char) and 5 % (digestate) as well as 5 % (char) and 5 % (digestate) were used. All mixtures prepared (soil- char and/or digestate) are based on percentage dry weight. From the literature study, it has been revealed that biomixtures that were mixed on volumetric basis (straw: peat: soil = 2:1:1) entail around 20 % of organic matter (Tortella *et al.*, 2012). Taking this as reference it was calculated that 20 % of organic matter corresponds to 12 % of organic carbon present in these biomixtures (assuming organic matter contains 60 % of organic carbon on dry mass basis). The soil mixture with a maximum concentration of 30 % of digestate (40 % C_{org} (w/w) in our study entail 120 g organic carbon per Kg soil that gives also 12 % which therefore is in line with the value derived from the literature) (Tortella *et al.*, 2012). For the char/soil mixtures the concentrations were decided on the basis of previous applications for pollutants studies (Yu *et al.*, 2010; Hailong *et al.*, 2010).

Measurement of soil microbial respiration (CO₂ release)

In order to analyze the degradation potential and dynamics of the organic substance within the soil/amendment mixtures respiration measurements with aliquots of the respective mixtures were accomplished. Therefore, 50 g aliquots (dry weight equivalent) of each soil treatment were taken and transferred into air tight bottles. In the respirometer device the evolved CO₂ as well as the consumed O₂ has been analyzed by an infrared gas analyser (Flow rate of gas -250 ml min⁻¹).

Due to the restriction of measuring 12 probes only at the same time within the given respirometer device all remaining samples were kept at 4 °C until first

measurement to slow down microbial activities.

For the respiration measurement the actual samples were disconnected from the respirometer and stored at the same temperature while the next set of 12 samples was attached to the device for 24 hours. Overall, for each sample the CO₂ release was recorded 8-10 times within the given 24 hours. With respect to the turnover of samples within the respirometer device soil respiration rates of the respective identical aliquots could be measured every 10 days. The temperature during respirometer measurement and storage was 20 ± 5 °C.

From the data of evolved CO₂ the arithmetic mean and the standard deviation of the respiration rate for each triplicate were calculated for each consecutive measurement date. Overall, the incubation time was 90 days for all samples.

Calculations and statistical interpretation

Kinetics of the carbon turnover

The course of the CO₂-C evolution from the soil and soil mixed with the organic amendments (biochar and digestate) was fit to different kinetic models with increasing pool complexity. In a first step, a single decaying carbon pool (SDCM) was assumed and the corresponding CO₂-C efflux over time t [d] can be described by:

$$C_t = C (1 - e^{-k \cdot t}) \quad [1]$$

where C_t is the mineralized carbon (CO₂-C) [g CO₂-C g⁻¹ mixture], C is the total amount of the carbon pool [g C g⁻¹ mixture], whereby the labile and refractory C-pool was bulked into one C-pool, and k is the first order mineralization rate of the carbon pool [d⁻¹].

Secondly, a double first order in parallel model (DFPM) was fitted to the observed data [Eq. 2] with

$$C_t = C_1 (1 - e^{-k_1 \cdot t}) + C_2 (1 - e^{-k_2 \cdot t}) \quad [2]$$

where k_1 and k_2 are the apparent first order mineralization rates [d⁻¹] of the labile and refractory C-pools, respectively, and C_1 is the amount of the relatively labile mineralizable carbon source and C_2 is the amount of relatively refractory mineralizable carbon source [g CO₂-C g⁻¹ mixture], respectively.

Half-life (HL) for the corresponding carbon pools can be calculated from the first order mineralization rates, k , by:

$$HL = \frac{\ln(2)}{k} / 365 \quad [3]$$

Statistical Analysis

The parameters providing the best prediction of the measured data were determined by minimizing the sum of squared residuals

$$SSR = \sum_{i=1}^n (x_{obs,i} - x_{sim,i})^2 \quad [4]$$

Where X and x_{sim} are the observed and simulated cumulative CO_2 -C fluxes [$g CO_2$ -C g^{-1} mixture] at time step i and n is the total number of observations. For the minimization of the objective function [Eq. 4] the global optimization routine shuffled complex evolution developed at the University of Arizona (SCE-UA) was used. This optimization routine has been already successfully applied in a wide range of applications in hydrology but also for the estimation of parameters in non-linear carbon models.

To quantify the quality the agreement between measured and fitted data of the inversion the coefficient of determination R^2 was calculated:

$$R^2 = \frac{\sum_{i=1}^n (x_{obs,i} - \bar{x}_{obs})(x_{sim,i} - \bar{x}_{sim})}{\sqrt{\sum_{i=1}^n (x_{obs,i} - \bar{x}_{obs})^2 \sum_{i=1}^n (x_{sim,i} - \bar{x}_{sim})^2}} \quad [5]$$

where \bar{x}_{obs} and \bar{x}_{sim}

are the arithmetic mean of the measured and fitted cumulative CO_2 -C fluxes, respectively.

RESULTS AND DISCUSSION

Application of the organic residues to the soil resulted in a different behavior of soil respiration. In general, cumulative fluxes increase over time for all soil types and mixtures, whereby the increase flattens out over time. As expected, highest CO_2 -C release was measured for the application of 30 % digestate due to the large amount of fresh carbon added to the system for both the Kaldenkirchen and the Merzenhausen soil. Nevertheless, total cumulated CO_2 -C is slightly larger for the Merzenhausen soil mixture (in case of 30 % digestate) with 16.38mg compared to the Kaldenkirchen soil mixture with 14.67mg, whereby the difference is 11%. For the 5:1 and 5:5 digestate and high temperature biochar mixture the percentage difference is 10% and 19 %, and even the native soil without any addition of amendments showed a difference in CO_2 -C evolved of 51 percent (0.44mg for the Merzenhausen and 0.29mg for the Kaldenkirchen soil), irrespectively of the fact that the total carbon content of both soils is nearly the same with a TOC content of roughly 1%. Surprisingly, the difference in 5:5 low temperature biochar and digestate mixture is higher with 30 %. Additionally, the

5 % digestate and 5:1 low temperature biochar digestate mixture shows differences between the two soils with 29 and 27 % flux differences for the Kaldenkirchen and Merzenhausen soil, respectively.

On the other hand, the addition of the two biochars lead to slightly enhanced carbon turnover compared to the native soil. For the Merzenhausen soil and Kaldenkirchen soil, total cumulative amount of CO_2 -C evolved are 0.47 ± 0.14 mg and 0.31 ± 0.093 mg respectively. With the addition of maximum amount of both high and low temperature biochar (5 % w/w) cumulative evolution of CO_2 -C becomes 0.84 ± 0.30 mg and 0.55 ± 0.17 mg (for Merzenhausen soil) and 0.72 ± 0.24 mg and 0.70 ± 0.20 mg (for Kaldenkirchen Soil). So, the percent increase of CO_2 -C evolution in both soils after addition of biochar varies between 17 (low temperature) -132 (high temperature)%. The increase of CO_2 -C evolution indicates presence of labile or volatile fractions of carbon in the applied biochar.

The mixtures of biochar and digestate show an intermediate flux between the biochar and the digestate, whereby the fluxes are much lower compared to the single addition of digestate, even if the same amount of carbon (or even slightly larger due to the add-on of the biochar) was added to the system. Still, the exact mechanisms of suppression of CO_2 -C fluxes from 5 % digestate in addition of 1 % and 5 % char are unknown but the pronounced suppression of fluxes of CO_2 -C after addition of higher amount of biochar (from 1 % to 5 % w/w basis) into the digestate is detectable. The probable mechanism of low fluxes of CO_2 -C from mixtures could be due to higher cation exchange capacity (CEC) and microporosity of char which sequester readily available nutrients from digestate, and therefore, are not available for the microorganisms, leading to negative 'priming effect' or even toxic effect of the added biochar (Kuzayakov et al., 2000; Marchetti et al., 2012).

In a next step, we compared the percentage of degraded carbon for the Merzenhausen and Kaldenkirchen soil amendment mixtures, respectively.

For both soils the percentage of degraded carbon was highest for the addition of 5 % digestate compare to other compositions and higher carbon inputs lead to lower degradation ratios. The difference in percent C degraded between both soils in case of 5 % digestate is 30 %. In case of the Merzenhausen soil 5:1 high temperature char and digestate mixture shows higher rate of degradation than 30 and 15 % digestate addition. In case of the Kaldenkirchen soil the picture is quite opposite. From the observation it becomes clear that the specific rate of degradation (with respect to total C added

to the system) is highest for 5 % digestate although the absolute rate of degradation is highest in 30 % digestate in both soils due to highest amount of available C.

Based on the incubation results a mixture of low temp biochar and digestate might be the most suitable biofilter material because of the slow release of CO₂-C or buffering activity of the mixture. Nevertheless, the degradation as well as the sorption potential of this mixture has to be tested in the next future.

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