Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn

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ABSTRACT

Biochar addition to soil has been generally associated with crop yield increases observed in some soils, and increased nutrient availability is one of the mechanisms proposed. Any impact of biochar on soil organisms can potentially translate to changes in nutrient availability and crop productivity, possibly explaining some of the beneficial and detrimental yield effects reported in literature. Therefore, the main aim of this study was to assess the medium-term impact of biochar addition on microbial and faunal activities in a temperate soil cropped to corn and the consequences for their main functions, litter decomposition and mineralization. Biochar was added to a corn field at rates of 0, 3, 12, 30 tons ha^{-1} three years prior to this study, in comparison to an annual application of 1 t ha^{-1}.

Biochar application increased microbial abundance, which nearly doubled at the highest addition rate, while mesofauna activity, and litter decomposition facilitated by mesofauna were not increased significantly but were positively influenced by biochar addition when these responses were modeled, and in the last case directly and positively associated to the higher microbial abundance. In addition, in short-term laboratory experiments after the addition of litter, biochar presence increased NO_2+NO_3 mineralization, and decreased that of SO_4 and Cl. However, those nutrient effects were not shown to be of concern at the field scale, where only some significant increases in SOC, pH, Cl and PO_4 were observed.

Therefore, no negative impacts in the soil biota activities and functions assessed were observed for the tested alkaline biochar after three years of the application, although this trend needs to be verified for other soil and biochar types.

1. INTRODUCTION

Biochar is a carbon(C) -rich product obtained by thermal decomposition of biomass at relatively low temperatures (<700ºC) and low oxygen concentration, in a process known as pyrolysis. During this process heat, flammable gases and liquids are produced together with a solid residue, biochar. The process resembles traditional charcoal production, but biochar is used as a soil amendment and not for energy generation (**Lehmann and Joseph 2009**). More recently, biochar has been more narrowly defined in terms of its capacity to sequester C and improve soil functions (**Verheijen et al. 2010**). Due to its particulate nature and its chemical structure, biochar is more stable than any other organic amendment which provides high recalcitrance to microbial decomposition (**Spokas 2010**), which has led to the consideration of biochar production as a C-negative technology for climate change mitigation (**Woolf et al. 2010**). Biochar application to soil and knowledge of its benefits to improve soil fertility is not new and has been practiced in traditional agriculture in many regions (**Ogawa and Okimori 2010**). However, the recent activity in biochar research and development has generated broad interest that has lead to a rapid spread of the technology.

Biochar is able to improve soil fertility in some soils (**Verheijen et al. 2010, Jeffery et al. 2011, Kookana et al. 2011, Spokas et al. 2012, Biederman and Harpole 2013**) as a result of its effects on physico-chemical and biological properties. Biochar has been shown to improve water retention, aggregation and permeability in some soils (**Downie et al. 2009, Busscher et al. 2010, Liu et al. 2012**), or increase the pH of acid soil (**Jeffery et al. 2011**), as well as increase plant nutrient availability in nutrient-limited agroecosystems (**Major et al. 2010**). Various mechanisms have been suggested for the latter such as: (1) the initial addition of soluble nutrients contained in the biochar **(Sohi et al. 2010)** and the mineralization of the labile fraction of biochar containing organically bound nutrients (**Lehmann et al. 2009**); (2) reduced nutrient leaching due to biochars' high cation exchange capacity (**Liang et al. 2006; Cheng et al. 2008,**

Laird et al. 2010, Spokas et al. 2012); (3) lower gaseous N losses by ammonia volatilization (**Taghizadeh-Toosi et al. 2012**) and N_2 and N_2O by denitrification (Cayuela et al. 2013); and (4) a retention of N, P and S associated with the increase in biological activities and/or community shifts (**Pietikäinen et al. 2000, Thies and Rillig 2009, Lehmann et al. 2011; Güereña et al. 2013**). Some of these mechanisms involve soil biota, and this is why effects on soil fauna might translate into changes in nutrient availability (**Altieri 1999, Lavelle et al 2006**). Despite this fact, effects on soil biota are one of the most understudied topics in biochar research (**Lehmann et al. 2011**), and many of the observed effects may be explainable with changes in soil biota.

In agroecosystems decomposer microorganisms are essential for nutrient release from soil organic matter to sustain crop production in addition to the inputs of fertilizers (**Bardgett 2005**). If biochar causes shifts in microbial communities, C cycling can also be affected (**Nielsen et al. 2011**), as well as other nutrients, and influence primary production or the fauna relying on microbiota. Not only changes in microorganism activity, but that of any soil biota group may have effects on other groups due to the complexity of below-ground food webs (**Bardgett 2005**). Therefore, an understanding of biochar effects on the interaction between a range of soil biota groups is needed.

Research on the effects of biochar on soil biota has been largely restricted to soil microbial abundance and activity. The change of the physicochemical environment, such as increased water and nutrient retention, and the provision of a refuge habitat protecting microorganisms from predators have been proposed as mechanisms (**Lehmann et al. 2011, Ennis et al. 2012**). However, studies on the impact on other biological groups are scarce in the scientific literature, especially with respect to soil fauna (**Lehmann et al. 2011**). In addition,the consequences of such impacts on soil functions such as decomposition and mineralization are poorly understood. It has been hypothesized that biochar might positively affect soil biota through the increase in soil aggregation and porosity, pH, moisture retention and soil temperature, as well as

nutrient retention (**McCormack et al. 2013)**, although negative effects might be also be expected with an enhanced retention of toxic substances, such as ammonium and pesticides (**Ennis et al. 2012, McCormack et al. 2013**), and the release of pollutants from biochar, such as pyrolysis oils (**Gell et al. 2011**) and PAH (**Hale et al. 2012)**. Currently there is a need for demonstration of the environmental benefits of biochar while avoiding detrimental effects on environmental health (**Verheijen et al. 2010**). Some biochars might pose a direct risk to soil biota and their functions (**Liesch et al. 2010, Weyers and Spokas 2011**), and may explain some of the negative crop yields reported in literature (**Spokas et al. 2012**).

The aim of our study is assessing the medium-term effects of biochar additions on microbial and faunal activity and their main soil functions, decomposition and mineralization.

2. METHODS

2.1. Experimental plots

The experimental plots were located at Cornell University's Musgrave Research Farm in Aurora, NY, USA (42º43'48.64"N, 76º39'16.03"W), continuously cropped to corn for more than 30 years in a soil and with an experimental design described in detail by **Güereña et al.** (**2013**). The experimental site was divided into plots of 4.5 x 7.5 m (33.7 m^2), with a 2-m buffer strip between them. Three plots were prepared per biochar addition rate in a completely randomized design. In April 2007, biochar was applied before planting, at rates of 0, 3, 12, 30 t ha^{-1}. In addition, an annual application of 1 t ha^{-1} was tested using the same batch of biochar (applied in 2007, 2008, and 2010, but not in 2009). Biochar was incorporated to plots by hand rake and shovel to a depth of approximately 50 mm which was then followed by mechanical tillage to about 0.13 m uniformly for all treatments.

The biochar was produced from corn stover by slow pyrolysis (30 min, 600ºC) at BEST Energies Inc. (Somersby, Australia), and its properties are described in **Güereña et al.** (**2013**). The ecotoxicological characterization of this biochar demonstrated no inhibition for the reproduction of soil collembolans (**ISO 1999**) and enchytraeids (**ISO 2004**) in soil-fresh biochar mixtures (0.2 to 14%, w/w) after 28 d of exposure (data no shown).

In the 2010 growing season of this study, three years after the application of biochar, a NPK fertilizer (10-20-20) was applied at planting (mid-May) at a rate of 12.3 kg N ha^{-1}. Three weeks after planting (early July), a secondary fertilization was applied at rates of 100.8 kg N ha^{-1} (corresponding to 90% of the recommended N application rate).

Plots were sown with a maize crop (Pioneer Hybrid 38M60 Triple stack, Pioneer Hi-Bred International, Inc., Johnston, IA, USA), at a rate of 79,287 seeds ha^{-1}. No pesticides were applied that year with the exception of pre-emergence herbicides applied just after sowing (atrazine and Lumax®), since a genetically modified and insect resistant corn variety was used. Exposure to genetically modified corn in field conditions has not been linked to detrimental effects on soil invertebrates or functions such as decomposition (**Cortet et al. 2006, Hönemann et al. 2008, Tarkalson et al. 2008**).

2.2. Soil physicochemical properties

Soil sampling was performed in summer 2010, three weeks after the secondary fertilization (late July), and in early fall (late September), which corresponded to the initial growth and the senescence of corn plants, respectively. Samples were taken in the four central rows of the plot using a metal core with a diameter of 45 mm diameter and length of 0.1 m. Three composite samples were taken per plot, each obtained from three soil cores.

The soil particle-distribution and texture were assessed in air-dried samples by the pipette method (**Gee and Bauder 1986**). The soil organic C (SOC) content was measured by the Walkley-Black procedure (**Nelson and Sommers 1982**). This method does not fully reflect C content of biochars (**Manning et al. 2009**), but the more labile fraction (**Calvelo Pereira et al. 2011**), hence potentially quantifying the most biologically relevant C fraction of biochars, potentially mineralizable by microorganisms which in turn could also affect other biological groups and soil functions.

The remaining soil properties were measured in an aqueous extract, where 25 g of fresh soil were mixed with 100 ml of deionised water and horizontally shaken at 160 rpm for 30 min. After that, soil particles were left to settle for 1 min, and the liquid phase was centrifuged for 5 min at 3600xg. Then the supernatant was gravimetrically filtered (Whatman 1). Half of the extract was used for immediate measurement of pH and electrical conductivity (EC), while the other half was used for quantification of the ionic content (NO_2, NO_3, NH_4, PO_4, SO_4 and Cl). For practical reasons, the extract for the last analysis was stored at -20ºC just after its preparation until the day of the analysis. Simultaneously, 20 g of the same fresh soil was weighed and dried at 105ºC for 12 h for assessment of the moisture content. Soil pH and EC were measured by potentiometry in an Orion 3-Star pH meter and an Orion 115 Aplus Conductivity Meter (Thermo Scientific Waltham, MA, USA), respectively.

In the summer sampling, soluble NO_2, NO_3,Cl and SO_4 were assessed by ionic chromatography (RFC 2000, Dionex) while $PO_4$and NH_4 were measured using a flow analyzer (FS 3000, OI Corporation) by the ascorbic acid and ammonium molybdate method (**Murphy and Riley 1962**), and the phenate method (**APHA 1985**), respectively. In the fall sampling, all the ions were measured by ionic chromatography (DX-100, Dionex).

2.3. Microorganism abundance, activity and efficiency

In summer 2010 (early July), fifteen soil cores with a diameter of 45 mm and a length of 0.1 m were taken per plot and stored separately. In the laboratory, composite samples were prepared, each containing three randomly selected cores, thereafter used for the assessment of microbial biomass (MCB) in duplicate, and the soil basal respiration (BAS) in triplicate.

MCB was taken as a measure of microbial abundance, and was measured by the fumigation-extraction method (**Brookes and Joergensen 2006**). The uncorrected MCB values were multiplied by a correction factor obtained from the dataset in **Jin (2010)**, in a study carried out in the same plots, to account for the underestimation of MCB due to the sorption of cell lysates to biochar (**Liang et al. 2010**). Namely, the correction factor was 1.53, 1.55, 1.62, and 1.77 for the plots with 0, 3, 12 and 30 tons ha^{-1} application rate, respectively, and 1.55 for the plots with the annual 1 t ha^{-1} application.

BAS was measured according to **Pell et al. (2006)** after 24 h of incubation at 20ºC, and taken as measure of total microbial activity. The C mineralization coefficient (CMC), expressed as the ratio of BAS to the summer organic C values was also calculated, and taken as a standardized measurement of microbial activity.

Microorganisms C-use efficiency was assessed by the metabolic quotient (qCO_2), obtained from the BAS/MCB ratio, which has been suggested as an indicator of the energetic efficiency of the community and hence of the succession and stabilization of the community after a disturbance (**Anderson and Domsch 1990**), as well a measure of microbial community stress (**Wardle and Ghani 1995**).

2.4. Fauna activity

Fauna feeding rates were assessed by the bait lamina method (**von Törne 1990**) using bait-lamina purchased from Terra Protecta GmbH (Berlin, Germany). The method is sensitive to variation in soil faunal activity after anthropogenic impacts such as pollution (**Filzek et al. 2004, Hartley et al. 2008**), or agricultural management practices (**Reinecke et al. 2008**). Some degree of microbial decomposition of the bait could be expected (**Von Törne 1990, Kratz 1998**), but it mainly reflects fauna feeding activity, such as that of collembolans and enchytraeids (**Helling et al. 1998, Gongalsky et al. 2008**), but also earthworms (**Van Gestel et al. 2003, Förster et al. 2004, Hamel et al. 2007, Gongalsky et al. 2008**).

Bait-lamina consisted of a 160-mm PVC stripe with 16 consecutive holes filled with a mixture of cellulose powder and bran flakes (7:3, w/w), and traces of activated carbon (**Kratz 1998**). Feeding activity was assessed as bait consumption one or two weeks after inserting it into the soil. Total feeding rates, as well as the depth-specific rates (0-30, 30-60, 60-80 mm-depth), were investigated. Feeding activity was assessed as qualitative feeding (percent of holes showing any degree of bait consumption) and as quantitative feeding (mean intensity of such consumption, visually assessed in each hole as 0, 10, 25, 50, 75 and 100%).

The summer sampling was carried out at the beginning of the cropping season (early July 2010), and the fall sampling at the end of the cropping season (late September 2010). In summer, seven bait laminae were inserted per plot in the four central interrows between corn plants, and removed 20 days later due to dry and hot weather during the first week. In fall, bait laminae numbers were increased to twelve per plot and removed after 7 days. After sampling, laminae were immediately transported to the laboratory and visually assessed.

2.5. Litter decomposition

Decomposition was assessed in 2-mm and 0.16-mm mesh litterbags, consisting of two 0.2x0.2 m squares, bent and stapled laterally to avoid litter losses. The 0.2-mm mesh corresponded to a regular PVC insect screen, while the 0.16-mm mesh corresponded to a polyester Accu-Mesh® 160 microns white screen mesh (Alpha Screens & Supplies Inc, Hicksville, NY). The 2-mm mesh bags assess decomposition resulting from the combined action of microorganisms, microfauna and mesofauna, while the 0.16-mm mesh only accounts for the decomposition due to microorganisms and microfauna (**Bradford et al. 2002**).

Each bag was filled with 5 g of corn stover, the same used for the mineralization tests, consisting of a mixture of leaves and stalks collected in the same plots in the 2009 harvest, then dried at 70ºC for 24h, and sieved to 15.9-4.76 mm to avoid losses through the bag's mesh. The use of corn stover was intentional in order to mimic the actual plant litter, as recommended for the litterbag method (**Knacker et al. 2003**).

In late June 2010, eight 50-mm deep soil holes were prepared in the four central interrows of each plot. In each hole, one 2-mm and one 0.16-mm mesh litterbag were buried side-by-side. After 3 months, all the bags were removed, hence covering most of the growing season of the crop, and immediately transported to the laboratory. Each litterbag was rinsed in tap water to remove soil particles, dried at 70ºC for 12 h and its content weighed.

2.6. Mineralization studies

Mineralization after litter addition to soil was assessed by adapting the OECD C mineralization test (**OECD 2000a**) and the N mineralization test (**OECD 2000b**), designed to assess the effects of pollution, to the purpose of this study, which was the

laboratory assessment of the C, N, P, S and Cl mineralization in soil samples taken from the field plots after the addition of corn stover.

Three soil cores (diameter of 45 mm and a length of 0.1 m) were randomly taken in each plot in late July and immediately transported to the laboratory. Soil samples were kept in the dark at 20ºC in sealed flasks, to avoid drying, and aerated twice per week to ensure oxygen supply, while a subsample of each soil column was used to assess their moisture and the maximum water holding capacity (WHC). After one week, soil column moisture was adjusted to 40% of the WHC. The day after, the same corn stover used in the litterbag experiment, but finely ground, was added at a rate of 0.15% (equivalent to a 3 t stover ha^{-1}), only slightly above the range of the stover inputs in corn crops reported by **Mann et al. (2002**). Then, nine subreplicates were prepared from each soil column to allow the destructive sampling of three replicates after 7, 14 and 28 days of incubation. Each replicate consisted of a 125 ml flask filled with 25 g of fresh soil. The CO_2 release in each subreplicate was measured by the method already described for BAS, and then the same subreplicate was used to prepare the aqueous extracts for the ionic content assessment. Water soluble NO_2, NO_3, NH_4, PO_4, SO_4, and Cl were measured by ion chromatography as described above. In order to avoid any bias of the initial mineralization products as well as its retention by biochar, mineralization was expressed as net mineralization rate. More precisely, for each mineralization product and replicate, concentrations were plotted against days of incubation and the slope obtained after linear regression was taken as mineralization rate. NO_2 and NO_3 concentrations were combined for the calculation of mineralization rates, since NO_2 is transient in soil under aerobic conditions and quickly converted to NO_3 (**Burns et al. 1996**).

Although mineralization products measured in our study might also come from native organic matter or from biochar mineralization itself (**Keith et al. 2011**), most of the

nutrients released should come from stover. Furthermore, we consider water extracts to be representative of the most bioavailable fraction for plants and soil biota.

2.8. Statistical analysis

All statistical analyses were carried out using R software version 2.15.0 (**R Development Core Team 2012)**. Several measurements were carried out within each plot, but only the mean value per plot was used for the statistical analysis, preventing pseudoreplication. A General Linear Model (*lm* function in R software), including biochar application rate as a factor, was used to test differences by biochar application rates compared to control plots, followed by a one-way ANOVA of this model to assess global differences (*anova* function of the R software). For variables with two sampling events, separate analyses were carried out for summer and fall data, as well for the mean annual values.

Pairwise correlations between measured response and explanatory variables were assessed by Pearson correlations (*cor* function in R software). For the annual biochar application of 1 t ha^{-1}, the value used for correlations was 3 t ha^{-1}, since this was the cumulative amount applied at the moment of the study since the first application in 2007.

The response variables (MCB, BAS, CMC, qCO_2, fauna feeding, decomposition and mineralization rates) were modeled using Generalized Linear Models (GLM) as a function of the explanatory variables (biochar application rate together with all the physical, chemical and biological soil properties measured). GLM were constructed using identity as link function, and assuming Gaussian distribution of the response variables (*glm* function of the R software). An initial global model including all the variables was constructed, and then all the possible models, restricted to three explanatory variables at most, were constructed and arranged from the best to the

worst goodness of fit (lowest AICc). AICc corresponds to a corrected Akaike information criterion, suitable for small sample sizes or high number of parameters in the model, which is the case of our dataset. This best model selection was carried out using the *dredge* function of the *MuMIn* package of the R software (**Bartoń 2007**).

3. RESULTS

3.1. Soil physicochemical properties

Particle-size distribution and soil moisture characteristics were not significantly affected by biochar additions irrespective of application rates, indicating a homogeneous texture in the experimental plots (**Supplementary Table S1**, **Figure 1**). SOC values were only significantly higher with an application rate of 12 t ha^{-1} in summer ($p=0.049$) compared to control plots, but not in fall. In summer, pH values were significantly higher at all biochar rates compared to control plots, while no differences were observed in fall (**Figure 1**). Acccordingly, a positive correlation between biochar rate and summer pH was observed (0.005 pH units per ton of biochar, $r=0.57$, $p=0.02$, data not shown). When the mean annual values were compared individually, only the annual 1 t ha^{-1} addition rate and the 30 t ha^{-1} addition rate showed significant pH increases (2.4 and 2.8%, respectively) compared to control plots (**Supplementary Table S1**). EC, NO_2, NO_3, NH_4 and SO_4values did not differ in biochar-amended plots compared to control plots, neither in summer or fall (**Figure 1**), nor in the mean annual values (**Supplementary Table S1**), but increased PO_4 was observed in the fall sampling with annual biochar applications (**Supplementary Figure S1**). Furthermore, significant positive correlations were detected between biochar application rate and summer PO_4 and fall Cl ($r=0.74$ and 0.61 respectively, data not shown).

3.2. Microorganism abundance, activity and efficiency

BAS, CMC and qCO_2 did not significantly vary with different application rates when compared to control plots. MCB, however, was significantly higher with an addition of 30 t ha^{-1} biochar compared to controls (p=0.03) (**Table 1, Figure 2**), and was positively correlated with biochar application rates (r=0.60, p=0.01, data not shown). However, we cannot discard significant changes in CMC and qCO_2, since the small sample size and high within-group variability may cause a type II error, i.e. failure to reject the null hypothesis that the means of the groups are equal when the alternative hypothesis is true.

The models for logMCB, BAS, CMC and qCO_2 accounted for 56, 62, 14 and 68% of the observed variance, respectively. The model derived for MCB only included moisture as an explanatory parameter (**Supplementary Table S2**), indicating higher MCB with higher moisture, while biochar application rate was not included. In the model for BAS only soil texture was included, while the model derived for CMC was not acceptable due its low predictability and because the only parameter included was not significant by itself. Finally, the model for qCO_2 included moisture and SOC as positive parameters, and sand content as a negative parameter, although SOC was not in itself significant.

3.3. Fauna feeding activity

No significant differences in the feeding rates were found between biochar-added plots and controls, irrespective of season (**Figure 3**, **Table 1**) nor were correlations with rates of application significant (**Supplementary Table S3**). Although a type II error might be also suspected due to the high variability in this response, modeling of summer feeding rates showed that only soil texture, together with other physicochemical properties, explained the variation in feeding rates observed between

plots but not biochar additions (**Supplementary Table S4**). Hence, feeding was almost entirely explained by soil texture, with the only exception of the qualitative summer feeding rates at 30-60 mm-depth, which appeared to be also positively influenced by MCB and soil SOC, and negatively by PO_4^{3-}. The models derived for summer feeding rates explained between 52 and 77% of the variance observed. In the fall feeding rates, more consistent trends were found, with a positive effect of biochar in some of the models (although this parameter was not significant by itself in some cases) and a general negative contribution of loam contents (**Supplementary Table S4**). The models for fall feeding rates explained between 53 and 69% of the variance.

3.4. Litter decomposition

No differences were found in decomposition rates assessed with litter bags whether or not biochar had been added to soil, for any of the mesh sizes (**Figure 4, Table 1**), and no direct correlations were found between decomposition and biochar addition rates, probably related to the high variability in these response that makes a type II error plausible (**Supplementary Table S3**). In the 2-mm mesh bags, significant positive correlations were found between decomposition and logMCB (r=0.59), and a negative correlation with qCO2 (r=-0.71) (**Supplementary Table S3**). However, when the 2-mm mesh bags decomposition was modeled, biochar and pH were shown to have a positive effect on this response variable, and Cl a negative effect (explaining 72% of the variance;**Supplementary Table S5**). In the 0.16-mm mesh bags, the decomposition model, explaining 74% of the variance, included a positive contribution of logMCB, clay and SOC, but not of the biochar application rate (**Supplementary Table S5**).

3.5. Mineralization studies

Positive mineralization rate values indicate an increase of mineralization products over time, while negative values indicate a decrease, in relation to the initial contents. None of the mineralization products assessed showed significantly different values in biochar-added plots compared to controls, with the exception of Cl and SO_4, showing negative rates in all the biochar addition rates and the 30 t ha^{-1} addition, respectively (**Figure 5**). In NO_2+NO_3 and PO_4 mineralization, the lack of significant effects might be also due to a type II error. However, when correlations with biochar addition rate were sought, only a positive correlation with NO_2+NO_3 mineralization rates was found (**Supplementary Table S3**). The models derived for the mineralization rates also showed a general lack of effect of biochar addition rate (**Supplementary Table S6**). Models derived for PO_4 and NO_2+NO_3 showed very low predictability. Only the models obtained for Cl, NH_4, SO_4 and CO_2, showed a relatively high predictability (76, 70, 57 and 51% of the variance observed), some including pH as a negative parameter (Cl and SO_4 models) and soluble PO_4 as a positive parameter (Cl and NH_4 models), but also MCB in the case the CO_2 and NH_4 models.

4. DISCUSSION

4.1. Biochar effects on soil biota

4.1.1. Effects on microbial abundance, activity and C-use efficiency

The increased microbial abundance after three years of biochar additions is in accordance with a study carried out in the same plots after 6 months of the application (**Jin 2010**) and with other published studies (**Lehmann et al. 2011**). Several hypotheses have been proposed to explain this fact, such as an enhanced habitat

suitability and refuge (**Pietikäinen et al. 2000, Warnock et al. 2007, Thies and Rillig 2009**), less competition (**Lehmann et al. 2011**), higher availability of nutrients or labile organic matter on biochar surfaces (**Pietikäinen et al. 2000, Bruun et al. 2009, Lehmann et al. 2011**), positive priming (**Zimmerman et al. 2011**), or changed physical properties that increased water retention and aeration (**Wardle et al. 1999, Schimel et al. 2007, Thies and Rillig 2009, Lehmann et al. 2011**). In our study, moisture was the main explanation for increased MCB, as shown by its strong positive correlation with biochar application rates (r=0.75, data not shown), as well as by the model derived for MCB, where soil moisture is the only parameter included (**SupplementaryTable S2**).

In contrast, the absence of changes in microbial activity, when measured as BAS, indicates that net microbial processing of organic C did not change with application of biochar but rather with differences in soil texture. This result is in agreement with other long-term studies under field conditions were no change or even lower respiration rates were observed in biochar-amended plots, and contrasts with short-term effects just after the application of biochars, generally associated to increased respiration rates associated with the easily mineralizable organic content of fresh biochars (see **Lehmann et al. 2011, Woolf and Lehmann 2012** for reviews on this topic).

The positive correlation of decomposition in the 2-mm mesh bags with MCB (in turn positively explained by biochar application rate) and the negative association with qCO_2 (**Supplementary Table S3**), though not directly linked to biochar application rates, suggest shifts in the microbial community composition to more efficient communities that favor decomposition. This is corroborated by a previous study carried out in the same plots shortly after the biochar addition (**Jin 2010**), where an increased abundance of highly efficient decomposers such as fungi was observed. Although we lack direct data, this explanation might be coherent with the inclusion of pH as being important to decomposition in the 2-mm mesh bags, since the liming effect of biochars has been suggested to cause a shift to lower bacteria-to-fungi ratios therefore favoring

fungivore fauna over microbivore ones (**McCormack et al. 2013**). Even though soil in our plots already had a pH around 7, it is interesting to note that lower bacteria-to-fungi ratios were found in the 12 and 30 t ha^{-1} plots in a previous study carried out one year after biochar application (**Jin 2010)**.

4.1.2. Effects on fauna activity

Fauna feeding activity was not directly affected by biochar applications, in spite of the observed changes in both microbial biomass and soil pH. Higher microbial biomass is expected to translate into increased microbial grazer populations and from them to predators, as shown in microcosm experiments (**Cole et al. 2004**). Similarly, a potential stimulation of soil fauna with pH increase after biochar addition suggested by **McCormack et al. (2013)** is also limited in our plots due to the already relatively high pH of the soil in this study (pH=7). On the other hand, excessive increases in pH might reduce the abundance of faunal groups such as collembolans, mites or earthworms and enchytreids and change the entire soil community and their functions (**Bardgett 2005**, **McCormack et al. 2013**) which again does not seem to be the case for our plots. Accordingly, no significant variation in fauna activity was observed with corn biochar additions, and any observed changes were mostly related to soil texture. Coarse soil particles and soil particle aggregation processes are directly related to soil porosity, a key property for fauna movement and performance in soil (**Lavelle et al. 2006)**. Also, microbial biomass was included as a positive parameter in one of the summer models, as well as biochar rate in some of the fall models, which suggests that soil fauna activity increases are also partly explained by increased food availability with biochar addition, since as previously reported, MCB was positively correlated with biochar addition.

4.2. Biochar effects on biota-mediated soil functions

4.2.1. Litter decomposition

The observed lack of direct effects of biochar on field litter decomposition and laboratory C mineralization after the addition of litter is consistent with the general lack of effects on microbial and faunal activities three years after application of biochar. Similarly, the model for microbial and microfauna decomposition without the regulatory effect of mesofauna (that of the 0.16-mm mesh litterbags), did not include biochar, but included a positive contribution of MCB and SOC, which in turn we observed to be associated with the biochar addition application rate. In contrast, the model for decomposition rates with access by mesofauna (2-mm mesh litterbags), included biochar addition as a positive parameter, which is not in agreement with the general lack of biochar effects on fauna activity. Therefore, a minor effect on enhancing litter decomposition by soil fauna through the presence of the tested biochar in our study cannot be excluded.

4.2.2. Mineralization

In contrast to our findings of increased N-mineralization with the increasing biochar addition in our aged biochar plot samples, decreased nitrate contents have typically been explained as a result of increased microbial biomass and N assimilation shortly after the application of fresh biochars with high labile C contents (**Bruun et al. 2009, Deenik et al. 2010, Clough et al. 2013**). The mechanisms for increased N-mineralization in our study are unknown, but this trend is probably transient and may be restricted to the conditions of a pot experiment, as this was not observed at the field scale. The observed lack of greater extractable NO_3 in the biochar-added plots at the field scale may be partly explained by biochar's poor anion retention (**Lehmann et al. 2003, Hale et al. 2013, Hollister et al. 2013**). Despite a typically high CEC of biochars

and observed N retention (**Steiner et al. 2008**), NH_4 did not accrue in topsoils which agrees with **Güereña et al. (2013)**, who quantified extractable mineral N in the same experiment in fall 2009. Even with lower N leaching (**Major et al. 2010**), extractable mineral N may not accumulate in soil, either because of greater plant N uptake (**Major et al. 2012**) or incorporation in organic N (**Güereña et al. 2013)**.

The influence of biochar on S and Cl-mineralization in soil has received little or no attention in literature (**DeLuca et al. 2009**) despite the role of such compounds in primary production (**McGrath et al. 1996, Öberg 2002**), and the decreased S and Cl mineralization rates observed with biochar in this study have not been reported previously.

Regarding S mineralization, an increased S assimilation by microorganisms or shifts in microbial community composition could be potential explanations in our enclosed incubations (**DeLuca et al. 2009**), since fresh biochars have been shown to release significant amounts of soluble inorganic S (**Uchimiya et al. 2010, Churka-Blum et al. 2013**) and SO_4 retention in biochar is negligible (**Borchard et al. 2012**). Even though S is present as inorganic salts in fresh biochars and is readily released shortly after its addition to soil, S concentrations did not increase with biochar additions at the field scale after three years of the application, at least partly due to its ease of leaching.

The positive correlation with biochar application rates observed for Cl at the field scale (data not shown), is probably a result of the initial application of the corn stover biochar, typically containing significant amounts of Cl (**Johansen et al. 2011, Rahim et al. 2013**). Some studies have linked Cl addition to soil with nitrification inhibition (**Belser and Mays 1980, Bauhus et al. 1996**), but in our study the increased field Cl concentrations with biochar application were not related with a nitrification reduction. The same trend was observed in the laboratory, where nitrification rate was uncorrelated to Cl mineralization rates.

None of the short-term nutrient mineralization effects observed in the laboratory pot experiments were translated to differential soluble ion contents at the field scale, hence suggesting these effects to be transient or counteracted by other processes such as rainfall, plant uptake, or nutrient gaseous losses that also contribute to the observed soluble ion contents.

5. CONCLUSIONS

The medium-term effects of biochar on soil biota in the studied sandy loam soil in a temperate climate were restricted to the higher microbial abundance without increases in microbial activity, as already reported in the same plots shortly after the addition of biochar, although a positive contribution of biochar was also shown for mesofauna activity and litter decomposition facilitated by mesofauna after modeling of those responses. The observed changes in nutrient dynamics were likely related to salt effects in short-term laboratory studies which have significance for use of biochar in growing media or potting soil, but were not shown to be of concern under field conditions. The interactions between microbial dynamics and faunal activity warrants further research, and information about faunal abundance and composition may prove rewarding. No concern about the use of the tested alkaline biochar in the studied temperate soil emanated from the reported experiments after three years, but has to be verified for other soil and biochar types.

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Table 1. Summer microbial activity values, mean annual feeding rates, and litter decomposition rates after corn biochar additions to a temperate soil. BAS corresponds to the basal soil respiration expressed as µg C-CO_2 g^{-1} h^{-1}, MCB corresponds to the microbial biomass expressed as µg C g^{-1}, while the remainder values are expressed as percent; CMC corresponds to the carbon mineralization coefficient, expressed as µg C-CO_2 g SOC^{-1}; qCO_2 corresponds to the BAS to MCB ratio.

Biochar (t ha^{-1})	**Plot**	**BAS**	**MCB**	**CMC**	**qCO_2**	**Fauna feeding rate** (quantitative)	**Fauna feeding rate** (qualitative)	**Litter decomposition** (2-mm mesh bags)	**Litter decomposition** (0.16-mm mesh bags)
0	4	0.72	528.2	0.042	0.001	0.44	0.56	0.55	0.55
	8	0.61	243.3	0.037	0.003	0.27	0.39	0.50	0.54
	16	0.70	272.9	0.039	0.003	0.44	0.54	0.59	0.58
3 (1 per year)	11	0.52	287.9	0.020	0.002	0.43	0.57	0.55	0.57
	18	0.83	329.0	0.046	0.003	0.36	0.43	0.52	0.54
	31	0.67	558.1	0.033	0.001	0.32	0.39	0.56	0.56
3	6	0.78	380.9	0.031	0.002	0.44	0.56	0.52	0.51
	10	0.55	382.8	0.029	0.001	0.40	0.50	0.57	0.54
	35	0.99	318.9	0.039	0.003	0.27	0.38	0.51	0.55
12	1	0.72	201.6	0.041	0.004	0.43	0.55	0.46	0.48
	13	0.63	210.4	0.024	0.003	0.39	0.50	0.51	0.57
	29	0.67	430.28	0.022	0.002	0.41	0.53	0.57	0.60
30	14	0.68	350.5	0.039	0.002	0.52	0.66	0.53	0.57
	27	0.65	977.0	0.026	0.001	0.43	0.53	0.57	0.57
	36	1.06	864.1	0.049	0.001	0.39	0.50	0.56	0.60

Figure 1. Soil moisture in the summer sampling, and pH, electrical conductivity, and soil organic carbon in both summer (black bars) and fall (white bars) for different biochar application rates (0, 3, 12 and 30 t ha^{-1} added once four years prior to the study, and 1 t ha^{-1} added annually). Error bars correspond to the standard deviation ($p<0.05$, $n=3$), while asterisks indicate significant differences compared to the respective control plots (0 t ha^{-1}).

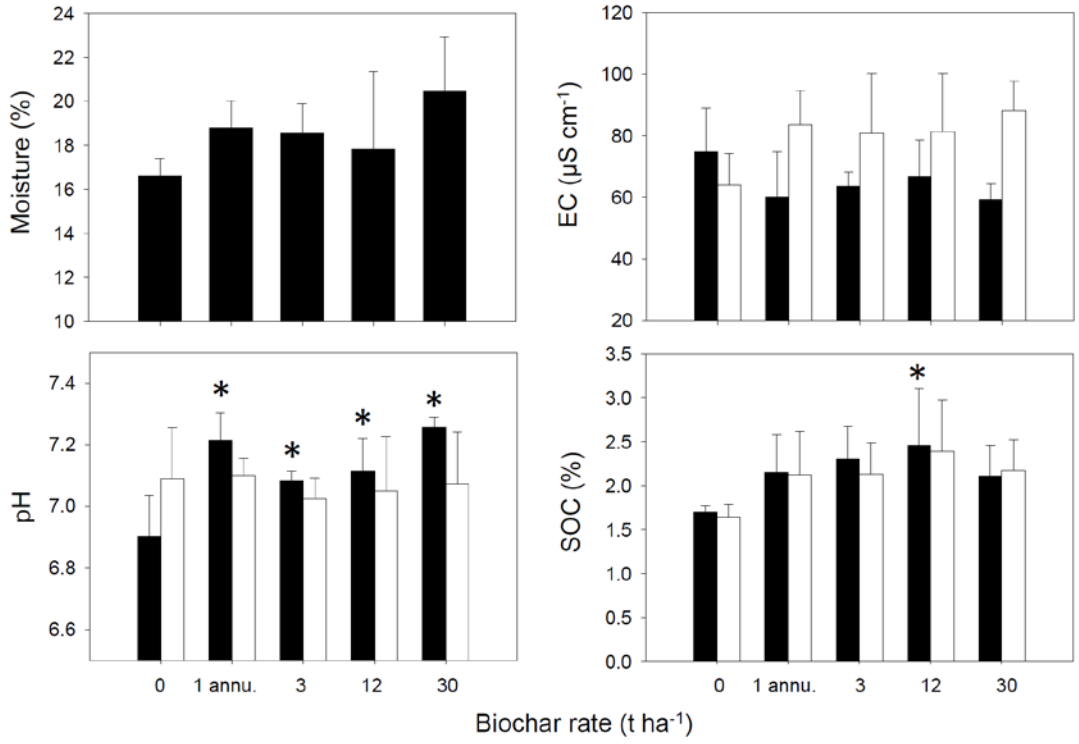


Figure 2. Microbial dynamics as affected by different biochar application rates. Error bars correspond to the standard deviation, while asterisks indicate significant differences compared to the respective control plots (0 t ha^{-1}) ($p<0.05$, n=3); *MCB*=microbial biomass, *BAS*=basal respiration, CMC= carbon mineralization coefficient; qCO_2=metabolic quotient.

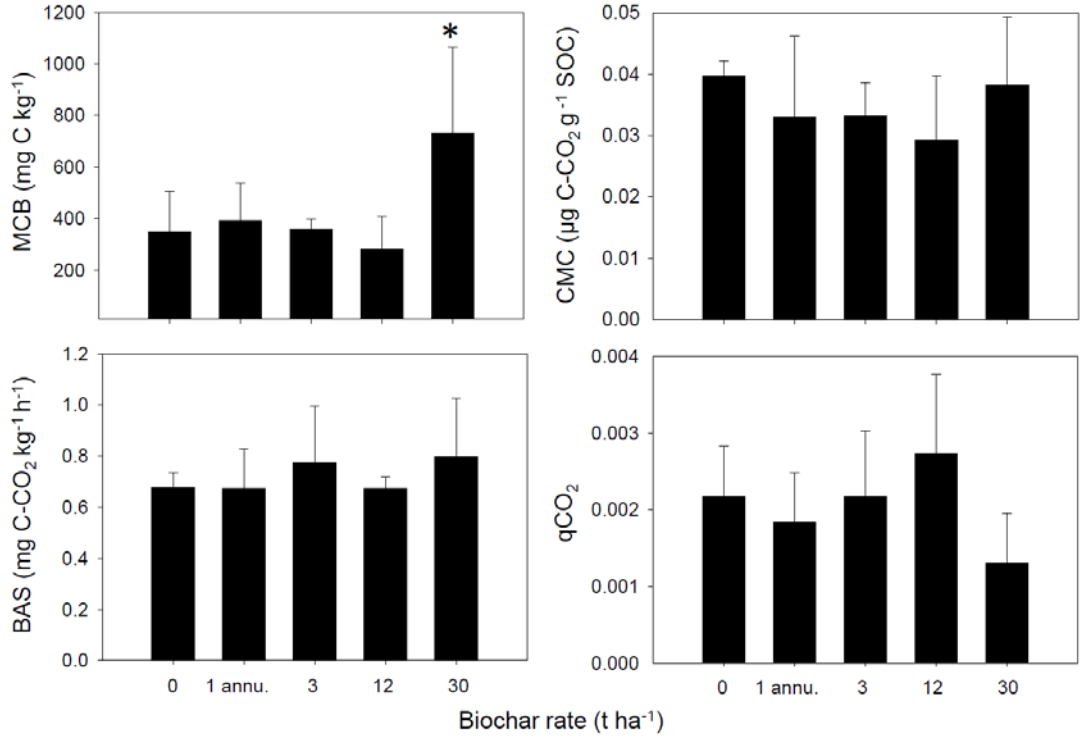


Figure 3. Fauna feeding activity, expressed as a rate, for the different biochar application rates in summer (black bars) and fall (white bars) for different soil depths. Error bars correspond to the standard deviation. No significant differences of the biochar-added plots compared to the respective controls (0 t ha^{-1}) were observed ($p<0.05$, $n=3$).

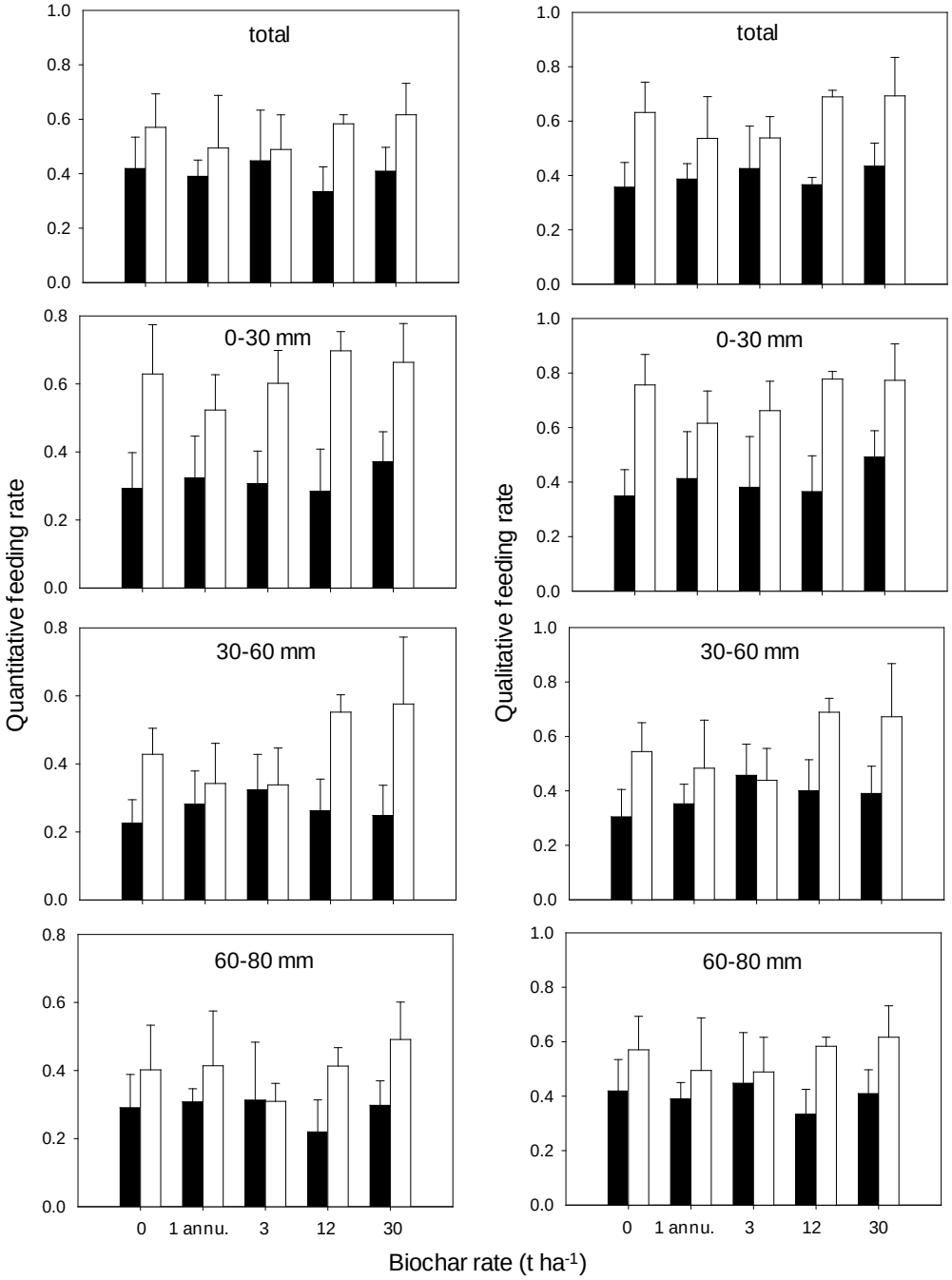


Figure 4. Litter decomposition rates for different biochar application rates as affected by microorganisms+microfauna+mesofauna (2-mmmesh litterbags) and microorganisms+microfauna (0.16-mmmesh litterbags). Error bars correspond to the standard deviation. No significant differences of the biochar-added plots compared to controls (0 t ha^{-1}) were observed ($p<0.05$, $n=3$).

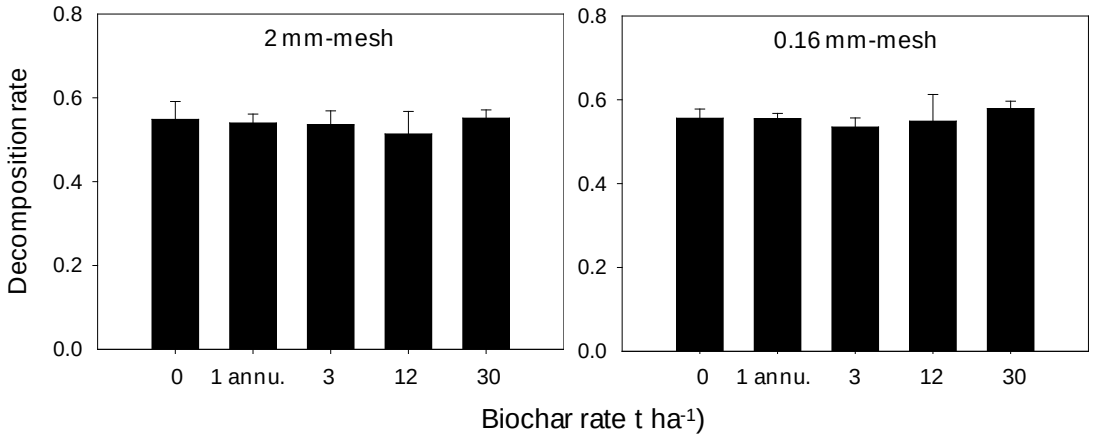
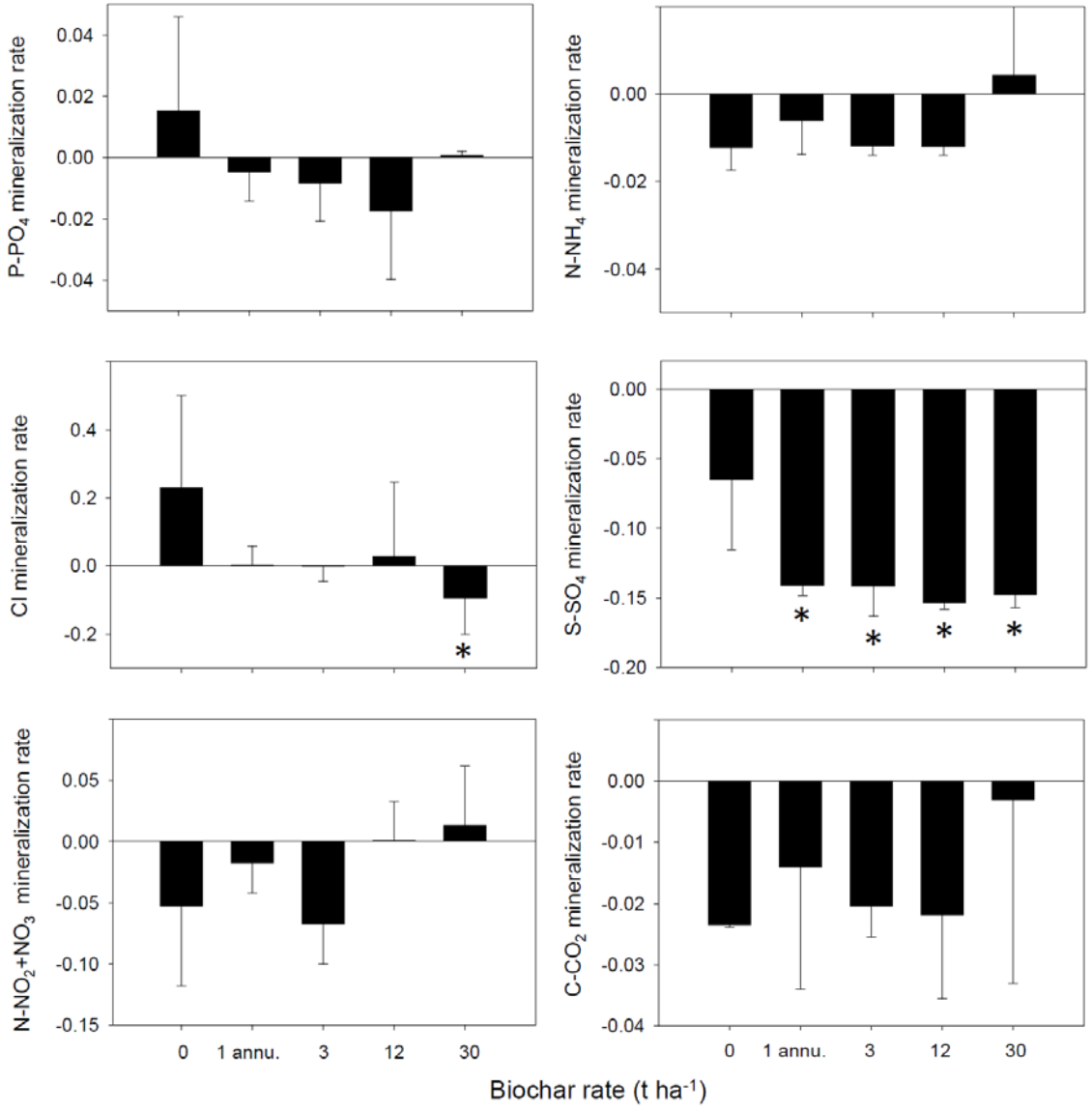


Figure 5. Mineralization and release rates of several ions during a 28-d incubation period, after the addition of corn litter to soil samples collected from plots with different biochar application rates. Rates are expressed as mg/kg·day, and negative values indicate a decrease in the ion content along the incubation. Significant differences in the rates of the biochar-added plots compared to controls (0 t ha^{-1}) are indicated with an asterisk ($p<0.05$, $n=3$).



Supplementary Online Material

Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn

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Table S1. Mean annual values for the physicochemical measurements in the different plots and treatments, all expressed on a dry weight basis.

Biochar t/ha	**Plot**	**Sand** %	**Silt** %	**Clay** %	**Moisture** %	**pH**	**EC** μScm^{-1}	**SOC** %	**P-PO4** $mg\ kg^{-1}$	**Cl** $mg\ kg^{-1}$	**N-NO2** $mg\ kg^{-1}$	**N-NO3** $mg\ kg^{-1}$	**N-NH4** $mg\ kg^{-1}$	**S-SO4** $mg\ kg^{-1}$
0	4	61.5	20.3	18.2	16.3	6.9	73.1	1.7	1.5	13.7	0.7	22.9	8.3	27.5
	8	62.2	20.2	17.6	16.1	6.9	64.5	1.6	1.2	16.3	1.2	19.5	9.7	43.0
	16	56.1	19.3	24.6	17.5	7.1	69.9	1.7	0.8	18.3	0.9	7.9	1.4	80.2
3 (1 per year)	11	62.7	19.4	17.9	17.4	7.1	72.4	1.8	1.9	22.3	0.9	9.1	9.3	138.9
	18	56.9	23.9	19.2	19.2	7.2	77.1	2.2	1.4	40.2	0.6	16.4	2.0	59.3
	31	59.5	24.8	15.7	19.7	7.1	71.7	2.4	1.0	22.4	0.2	7.6	0.9	114.4
3	6	68.7	16.7	14.7	17.0	7.0	64.8	2.2	1.6	14.3	0.9	14.6	8.5	41.0
	10	62.8	21.1	16.1	19.0	7.0	69.8	2.0	1.7	10.7	0.9	12.6	8.9	75.7
	35	56.3	26.0	17.7	19.6	7.1	79.1	2.5	2.5	19.2	1.1	7.8	0.2	137.4
12	1	60.0	22.0	18	15.0	7.1	66.2	1.7	4.3	29.2	2.5	7.1	13.1	130.2
	13	63.4	20.9	15.7	16.8	7.1	71.3	2.3	3.0	12.8	0.9	13.9	9.5	95.5
	29	58.3	18.7	22.9	21.8	7.1	90.4	2.5	1.3	22.4	0.6	15.3	0.4	115.4
30	14	63.1	15.3	21.6	17.8	7.1	70.1	1.7	1.3	21.5	1.1	11.8	9.8	89.1
	27	59.1	22.9	18	22.7	7.2	58.8	2.8	1.0	29.9	1.0	9.7	1.3	26.6
	36	58.2	25.0	16.9	20.9	7.2	82.4	2.8	2.5	19.0	1.4	8.5	0.4	136.2

Table S2. Generalized linear models with best goodness of fit (lowest AICc) of microbial measurements using explanatory variables from the summer sampling, when those microbial measurements were carried out. All the parameters are significant in itself in the model except those indicated as (ns).

Response variable	**Explanatory variable coefficients**								
	intercept	*moisture*	*sand*	*loam*	*clay*	*SOC.summer*	*NO_3.summer*	*AICc*	*r^2*
logMCB	1.315	0.068						-10.6	0.56
BAS	-229.2		2.287	2.317	2.316			-13.9	0.62
CMC	0.440						-0.006 (ns)	-19.5	0.14
qCO2	0.017	0.0004	-0.0001			0.0008(ns)		-178.8	0.68

Table S3. Pairwise Pearson correlation coefficients of the biological responses assessed. Fauna feeding rates correspond to the mean of the summer and fall values. Significant relationships are indicated by coefficients highlighted in bold and asterisks indicating the significance (*=p≤0.05, **=p≤0.01); n=15.

	Biochar rate	**Fauna feedingrate (quantitative)**	**Fauna feedingrate (qualitative)**	**Decomposition (2mm-mesh)**	**Decomposition (0.1mm-mesh)**	**BAS**	**CMC**	**logMCB**	**qCO2**	**PO4 mineralization**	**Cl mineralization**	**NO2+NO3 mineralization**	**SO4 mineralization**	**NH4 mineralization**	**CO2 mineralization**
Biochar rate	1.00														
Fauna feeding (quantitative)	0.42	1.00													
Fauna feeding (qualitative)	0.43	**0.97****	1.00												
Decomposition (2mm-mesh)	0.06	0.23	0.17	1.00											
Decomposition (0.1mm-mesh)	0.36	0.03	0.05	**0.69****	1.00										
BAS	0.23	-0.28	-0.27	-0.15	0.09	1.00									
CMC	0.06	-0.14	-0.18	**-0.23**	-0.19	**0.69****	1.00								
logMCB	0.50	0.07	0.03	**0.59***	0.44	0.30	0.09	1.00							
qCO2	-0.27	-0.17	-0.15	**-0.71****	-0.42	0.06	0.16	**-0.84****	1.00						
PO_4mineralization	-0.08	-0.17	-0.13	-0.27	-0.24	-0.19	0.22	-0.16	0.10	1.00					
Cl mineralization	-0.43	0.01	0.00	-0.03	-0.43	-0.01	0.36	0.03	-0.04	0.06	1.00				
NO_2+NO_3mineralization	**0.52***	0.23	0.16	-0.09	0.09	-0.07	-0.16	0.05	0.25	-0.14	-0.36	1.00			
SO_4mineralization	-0.42	0.16	0.16	0.31	-0.06	-0.12	0.24	0.08	-0.19	0.24	**0.77****	-0.37	1.00		
NH_4mineralization	0.51	0.13	0.04	0.35	0.21	-0.12	-0.21	**0.72****	**-0.54***	-0.09	-0.05	0.48	-0.06	1.00	
CO_2mineralization	0.38	-0.22	-0.23	0.15	0.18	**0.56***	0.39	**0.60***	-0.39	-0.10	0.02	0.18	-0.15	0.22	1.00

Table S4. Generalized linear models for fauna feeding rates, for the total values and those at different depths at the two sampling times (summer and fall), expressed as rate. All the parameters are significant in itself in the model except those indicated as (n.s.).Unacceptable models (with no significant parameters in itself included) are indicated as n.a.

Sampling	Response variable	Depth (mm)	Explanatory variable coefficients											
			intercept	*biochar*	*logMCB*	*sand*	*loam*	*clay*	*SOC*	*PO4*	*NO2*	*NO3*	*Cl*	AICc	r^2
Summer	Qualitativefeedingrate	*total*	-142.98			1.435	1.421	1.444						-40.8	0.71
		0-30	-200.77			2.010	2.036	1.996						-21.7	0.55
		30-60	-0.55		0.322				0.127	-0.702				-22.3	0.54
		60-80	-176.93			1.776	1.757	1.782						-31.4	0.77
	Quantitativefeedingrate	*total*	-115.64			1.159	1.152	1.168						-34.3	0.55
		0-30	-156.11			1.561	1.558	1.584						-22.8	0.52
		30-60	0.412								-0.0841			-29.5	0.28
		60-80	-137.47			1.380	1.365	1.384						25.5	0.58
Fall	Qualitativefeedingrate	*total*	1.06	0.004(n.s.)			-0.023							-21.8	0.53
		0-30	1.281	0.003(n.s.)			-0.028							-33.4	0.69
		30-60	1.076				-0.034						0.005	-19.1	0.58
		60-80	n.a.												
	Quantitativefeedingrate	*total*	0.906	0.005			-0.021							-26.3	
		0-30	1.243				-0.027					-0.00914		-28.9	
		30-60	0.953	0.007			-0.027							-20.5	
		60-80	n.a.												

Table S5. Generalized linear models for litter decomposition, expressed as percent, derived using as explanatory variables the soil properties values measured in the fall sampling.

Response variable	**Explanatory variable coefficients**								
	intercept	*biochar*	*logMCB*	*clay*	*pH*	*SOC*	*Cl*	*AICc*	r^2
2-mmmesh litterbags	-1.235	0.002			0.259		-0.002	-62.7	0.72
0.16-mmmesh litterbags	0.179		0.063	0.007		0.044		-65.2	0.74

Table S6. Generalized linear models for litter mineralization rates, expressed as mg $kg^{-1} \cdot day^{-1}$, using as explanatory variables those collected on the summer sampling, when this assay was carried out. All the parameters are significantin itselfin the model except those indicated as (ns).

Response variable	**Explanatory variable coefficients**								
	intercept	*logMCB*	*moisture*	*loam*	*pH.summer*	*PO4.summer*	*Cl.summer*	*AIC*	*r2*
P-PO4	0.099		-0.005				-0.003	-74.2	0.46
Cl	9.645				-1.379	0.796		-24.1	0.76
N-NO2+NO3	0.039	-0.029(ns)				0.093		-55.8	0.38
N-NH4	-0.084	0.024				0.051		-103.7	0.70
S-SO4	1.321				-0.204			-61.9	0.57
C-CO2	-0.163	0.038		0.002(ns)				-84.3	0.51

Figure S1. Mean soil soluble ion content in summer (black bars) and fall (white bars), together with the standard deviation (n=3 plots). Asterisks indicate significant differences in the values measured in the biochar-amended plots compared to control plots (0 t ha^{-1}).