Biochar application and summer temperatures reduce N_2O and enhance CH_4 emissions in a Mediterranean agroecosystem: role of biologically-induced anoxic microsites

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ABSTRACT

Biochar applications have been proposed for mitigating some soil greenhouse gas (GHG) emissions. However, results can range from mitigation to no effects. To explain these differences, mechanisms have been proposed but their reliability depend on biochar type, soil and climatic conditions. Furthermore, it is found that the mitigation capacity is dependent on how the biochar is ageing under field conditions.

The effects on N_2O, CH_4 and CO_2 emission rates of a gasification pine biochar (applied as 0, 5, and 30 tonnes ha^{-1}) were studied between 8 and 21 months of the application in an alkaline soil cropped to barley under Mediterranean climate. Together with GHG, soil chemical and biological properties were assessed, namely, changes in labile organic matter content and nutrient status, and pH, as well as microbial abundance, activity, and functional composition.

During the 2 years of the application, significant changes were observed at the highest rate of biochar application such as higher contents of water, K^+, Mg^{2+}, SO_4^{2-}, higher basal respiration, and with non-significant changes in microbial community, though with some temporal effects. Regarding GHG, N_2O decreases coupled with CH_4 increases in the summer sampling were measured, although only for the highest application rate scenario. Such effects were unrelated to pH, bioavailable nitrogen status, or bulk soil microbial community shifts. We hypothesized that the key is the porous structure of our wood biochar, which is able to provide more and diversified microbial microhabitats in

comparison to bulk soil. At higher temperatures in summer, biologically-induced anoxic conditions in biochar pores acting as microsites may be promoted, where total denitrification to N_2 occurs which leads to N_2O uptake, while CH_4 production is promoted.

KEYWORDS: gasification biochar; microsites; methanotrophs; mitigation; N_2O, CH_4 and CO_2 gas exchange rates; N-cycle microbial functional groups.

1. INTRODUCTION

The increasing atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) associated with some human activities are of major concern due to their expected harmful impact on future climatic patterns. Soil is a key compartment regulating the cycling, production, consumption, and storage of these gases (Gärdenäs et al., 2010), with emissions regulated by a myriad of soil properties, processes and managements, such as the application of biochar assessed in this study.

Soil CO_2 emission mainly results from microbial and root respiration, and is mostly linked to temperature, water content, pH, nutrient and oxygen availability, as well as the quantity and quality of the organic materials concerned (Balogh et al., 2011; Xu et al., 2006). On the other hand, CH_4, with 25 times the global warming potential of CO_2 (IPCC, 2007), is produced under anoxic conditions, but in aerated and drained soils it is easily oxidized (Bodelier, 2011; Mosier et al., 1998). Nevertheless, methanogenesis may also take place under well aerated and drained soils by alternative metabolisms (Conrad, 2007) or microsites with anoxic conditions (Hagemann et al., 2016). Soil redox potential and substrate availability determine the balance between methanogenesis and CH_4 oxidation. Regarding N_2O atmospheric concentrations, with a global warming potential 298 times that of CO_2 (IPCC, 2007), it mainly results from the last step of denitrification, a process occurring in low oxygen environments and mostly carried out by heterotrophic bacteria able to reduce NO_3^- (nitrifying bacteria) to nitrous oxide (N_2O) and then to N_2 (Paul and Clark, 1996). This is why denitrification depends on NO_3^-, which is mostly an end product of the aerobic process of nitrification (Braker and Conrad, 2011). Both denitrification and nitrification are controlled by moisture, temperature, pH, and the availability of oxygen and metabolic substrates (Firestone and Davidson, 1989). In turn, the availability of CO_2 regulates nitrification, since most nitrifiers are chemoautotrophs, while the availability of organic C is essential for denitrification, as most of the denitrifiers are facultative anaerobic heterotrophic bacteria (Inglett et al., 2005, Levy-Booth et al., 2014). Other alternative processes are able to produce N_2O, such as nitrifier denitrification, chemodenitrification, and ammonia oxidation by methanotrophs (Bédard and Knowles,

88 1989; Heil et al., 2014; Kool et al., 2011; Wrage et al., 2001).

89 Biochar is a carbon-rich material intended to be used as soil amendment produced by
90 pyrolysis, i.e. thermal decomposition of biomass under a limited oxygen supply (Lehmann
91 and Joseph 2015). The broad variety of biochars in terms of physicochemical properties
92 mostly depends on the feedstock and production temperature used (Singh et al., 2012; Sohi
93 et al., 2010). Biochar's high sorption capacity and elevated recalcitrance to biodegradation
94 (Joseph et al., 2010; Keiluweit et al., 2010; Uchimiya et al., 2010, Uchimiya et al., 2012)
95 explain the current interest in these materials to improve soil nutrient retention and
96 pollution mitigation (Glaser et al., 2002, Major et al., 2010, Xiao et al. 2017), and carbon
97 sequestration (Goldberg, 1985; Laird, 2008; Lehmann, 2007). The liming capacity of some
98 biochars is also of interest in acidic soils (Van Zwieten et al., 2010), and more recently,
99 there is also interest in their potential role in the mitigation of soil greenhouse gases (GHG)
100 emissions (Major et al., 2010; Singh et al., 2010; Sohi et al., 2010; Spokas et al., 2012, Xiao
101 et al. 2017). The use of biochar for this purpose is heavily debated nowadays as an
102 integrative tool in agroecosystems (Woolf et al., 2010), since arable land can either behave
103 as a GHG source or as sink, contributing to 10-12% of the total annual GHG emissions
104 (IPCC, 2014, Erisman et al., 2011).

105 Biochar application has been shown to mitigate N_2O and CH_4 emissions from soils in some
106 studies (Cayuela et al., 2013; Feng et al., 2012; Karhu et al., 2011; Nelissen et al., 2014),
107 but not in others that failed to find any effect or that have demonstrated enhanced
108 emissions (Clough et al., 2010; Spokas and Reicosky, 2009; Van Zwieten et al., 2009;
109 Zimmerman et al., 2011). The biochar-mediated impacts on soil GHG production seem to
110 be either related to (i) biotic and abiotic processes intimately linked to the particular
111 biochar and soil considered (Atkinson et al., 2010; Lehmann et al., 2011; Shneour, 1966;
112 Spokas and Reicosky, 2009), or (ii) plausibly also to seasonality and the particular climatic
113 conditions of the site. The exact mechanisms responsible for these mitigation effects are
114 still unresolved (Lehmann et al., 2011; Warnock et al., 2007), although some authors have
115 recently argued that the impacts on soil microbial communities might be at least part of
116 the explanation (Khodadad et al., 2011; Lehmann et al., 2011). Microbial community

117 composition or activity shifts and the associated GHG emission changes, could be explained
118 by: (i) the changes in soil properties such as water availability, nutrient availability, and
119 pH buffering capacity and provision of electron donors (Ameloot et al., 2013a; Basso et al.,
120 2013, Harter et al., 2014), (ii) the creation of a more suitable habitat for microorganisms,
121 as biochar's high surface area and the refuge provided by its porosity against microbivores
122 (Quilliam et al., 2013), and (iii) the negative effects on particular microbial groups, such as
123 the demonstrated toxicity of compounds released by some fresh biochars able to inhibit
124 N_2O producing bacteria (Clough et al., 2010; Spokas et al., 2013).
125 Most studies have assessed the short-term effects of biochar on GHG, often under
126 laboratory conditions, despite the fact that biochar weathering under field conditions can
127 strongly change surface chemical functional groups and hence their effects on GHG over
128 time. Such changes are the result of abiotic processes (Cheng et al., 2006; Degroot et al.,
129 1991; Joseph et al., 2010; Puri et al., 1958) and biotic processes (Bird et al., 1999; Goldberg,
130 1985; Mul et al., 1998; Neeft et al., 1998; Watts, 1958). The limited attention in the
131 literature to this topic is surprising, given the critical interest for the duration of the GHG
132 suppression benefits under field conditions (e.g., Gaunt and Lehmann, 2008; Spokas et al.,
133 2012). Even more scarce are the studies carried out under Mediterranean climate and
134 alkaline soil conditions, with only one study available showing decreased N_2O emissions in
135 a wheat crop 3 months after the biochar application (Castaldi et al., 2011). Furthermore,
136 few studies have addressed the impacts of gasification biochars on GHG, despite their
137 particular physicochemical properties compared to slow and fast pyrolysis biochars (You et
138 al., 2017).
139 Therefore, the main objectives of this study were i) to describe the medium-term effects of
140 a gasification biochar, applied at low and high application rates, on N_2O, CH_4, and CO_2
141 emission rates in an alkaline Mediterranean soil. This will be observed under mesocosm
142 conditions and along an agronomical cycle 8 to 21 months after the biochar application,
143 taking special attention to describe changes related to different environmental conditions
144 over time; and ii) to relate such changes with chemical and microbiological properties to
145 reveal possible mechanisms behind the observed shifts in the emission patterns.

146

147 **2. MATERIALS AND METHODS**

148 ***2.1 Biochar and soil properties***

149 A pine chip (*Pinus pinaster* + *P. radiata*) gasification biochar was obtained from a pilot
150 gasification facility in Vitoria (Northern Spain). Details on its main properties are
151 described in Marks et al. (2014). The biochar's pH was 11.4, the electrical conductivity 644
152 µS cm^{-1} at 25° C, and ash and elemental C, N, S content of 9.49%, 88.41%, 0.30%, and
153 0.06%, respectively. Volatile matter (VM) was relatively low in this biochar (8%) in
154 accordance with the biochar's high production temperature (Enders et al., 2012). Loss on
155 ignition (LOI) at 375° C was moderate for a wood biochar (88%), which corresponds to the
156 organic matter content. LOI at 550° C was 0.73% and LOI at 1100° C was 1%, representing
157 the soot and carbonate contents, respectively. The relatively high content of carbonates
158 explains the high pH of this biochar (Enders et al., 2012).
159 The soil used in this experiment was collected from an experimental agricultural soil at
160 the Autonomous University of Barcelona campus (Cerdanyola del Vallès, Catalonia, NE
161 Spain) and corresponded to the top layer (20 cm) of a loamy Typic Calcixerept (**Table 1**).
162 The soil had been formerly used for grain production and no pesticides had been used for
163 at least 5 years. The high copper levels found in this soil were due to the prior use of copper
164 sulfate as a fungicide in the traditional vineyard cultures of this area, as found in many
165 soils in southern Europe (Brun et al., 2001), thus representing a realistic scenario.
166

167 ***2.2 Mesocosms setup***

168 Twenty-four field soil mesocosms, placed in the Autonomous University of Barcelona
169 Campus (41°29'53.34"N, 2°6'7.84"E) were installed on March 2011, each consisting of a 160
170 liters polypropylene box (53, 40.5 and 73 cm of inner height, width and length respectively)
171 with six holes (5 cm-diameter) in the bottom of the container that allowed proper drainage
172 of any excess water. To avoid soil loss through the holes, a plastic 2 mm mesh was placed
173 in the base of each container. The mesocosms were placed outdoors in two rows to enhance
174 their thermal isolation, and west-to-east oriented to ensure similar sunlight. We additionally

protected the boxes with a shading blanket. Each mesocosm was then filled with a 20 cm layer of unamended soil then covered by a 23 cm layer of soil (with or without biochar), which corresponded to 127 liters of uncompacted soil.

Unamended soil and two biochar addition rates were selected as treatments, consisting of a 0, 5, and 30 t biochar ha^{-1} application, respectively, each treatment being randomly assigned to eight microcosms out of twenty-four. These rates can be considered as low and high application rates within the range reported in biochar agronomic experiments (Jeffery et al., 2011, 2015). Due to the lower density of biochar compared to soil and to the volume-based criteria used to fill the boxes, the amount of soil-mixture contained in each mesocosm was slightly different depending on the treatment concerned, being 87, 85, and 77 kg of soil for the 0, 5, and 30 tonnes ha^{-1} of biochar, respectively.

A feed barley (*Hordeum vulgare* L.) of the variety Graphic (RAGT, Palencia, Spain), was annually seeded in the mesocosms in early January, after tillage, at a density of 300 seeds m^{-2} (116 seeds per mesocosm), and harvested in July. Pig slurry was added annually as fertilizer at the recommended dosage for this crop (100 kg N ha^{-1} $year^{-1}$), in a dosage calculated based on its hydrolyzable (labile) N content (see Marks et al., 2016). Half of the annual fertilization was carried out at seeding in early January, and the other half in mid-April when seedlings were growing vigorously, in agreement with the usual agricultural management practices in the area (Figure 1).

2.3 Soil chemistry

In fall 2011, and in spring, summer, and winter 2012 (and namely 8, 12, 16, and 21 months after the application of the biochar), soil samplings were carried out together with gaseous emission meaurements, and microbial community characterization. Soil samples of each plot were taken at 3 points with a core of 2.5 cm diameter auger at a 10 cm depth. The same day of the sampling, soil samples were immediately sieved (2 mm mesh) and homogenized. Water extracts (15 g soil:75 mL deionized water) were prepared and vertically agitated at 60 rev min^{-1} for 2 h, centrifuged, and filtered with Whatman #42 filter paper. The electrical conductivity (EC) and pH were determined immediately and the

extract was frozen at -20°C for determination of ion concentrations at a later date. Liquid ion chromatography was used to determine water-soluble concentrations of major cations and anions, simultaneously measured in a Dionex ICS-1100 ion chromatograph (Dionex, Sunnyvale, USA), being cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, and NH_4^+) assessed by means of a CS12A Dionex cation column on and anions (Cl^-, NO_2^-, NO_3^-, HPO_4^{2-} and SO_4^{2-}) by using a AS4A-SC Dionex anion column on a the same ion chromatograph.

2.4 Soil microbial biomass and activity

A 30 g aliquot of the sieved and homogenized soil samples used for the chemical assays above described was taken and stored at -20ºC for molecular analysis while another 30 g aliquot was immediately used for soil basal respiration and microbial biomass assessment. Soil basal respiration (BAS) was evaluated in gas traps following Pell et al. (2006). The same soil sample was then used to estimate microbial biomass by the fumigation-extraction method following Brookes and Joergensen (2006): two portions of the moist soil (15 g dry weight each) were weighed and noted as fumigated and non-fumigated sample batches. Finally, the metabolic quotient (qCO_2) was calculated as qCO_2 = (µg CO_2-C g $soil^{-1}$ $hour^{-1}$ / µg MBC g $soil^{-1}$) (Anderson and Domsch, 1990).

2.5 Soil microbial functional gene abundance

Soil DNA was extracted from soil samples using the MoBio ultraclean DNA soil kit (MoBio, Laboratories Inc., CA) as instructed by the manufacturer. DNA concentration and quality were spectrophotometrically assessed (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA), and by agarose gel electrophoresis.

Quantitative polymerase chain reaction (qPCR) was performed to assess the abundance of following genes: 16S rRNA for the total bacterial numbers, *amoA* for bacterial and archaeal ammonia oxidizers, *nirK* and *nirS* for nitrate reducers carrying a nitrite reductase gene, *nosZ* for denitrifiers carrying the N_2O reductase gene, and *pmoA* for methanotrophs carrying the methane oxidation. All the qPCR was conducted in 96 well plates using 7900HT Fast Real-Time PCR System (Applied Biosystems). The specific primer

combination and qPCR conditions used for each gene are shown in **Table S1**. Single PCR reactions were prepared in a total volume of 20 µl containing the following: 10 µl of SYBR Green qPCR Master Mix (Biotool), 0.5 µl of forward and reverse primer (10 µM) (Metabion); 0.5 µl of dimetyl sulfoxide, DMSO, (Sigma); 3.5 µl H_2O, and 5 µl template DNA (4 ng μl^{-1}). At the end of each run, melting curve analysis of the PCR products was conducted to confirm that the fluorescence signal came from specific PCR products and not from primer-dimers or other artifacts. Additionally, an agarose gel (2%) was run to check the correct size of amplicons.

The qPCR standards were obtained from the following sources: the field control soil gDNA (bacteria, bacterial *amoA* and archeal *amoA*); *Sinorhizobium melioti 1021* (*nirK* and *nosZ*); *Ralstonia eutropha H16* (*nirS*); *Acidithiobacillus ferrooxidans* (*nifH*) and *Methylomonas methanica* (*pmoA*). The PCR amplified DNA from the soil samples and the cultured microorganisms were purified using mi-Gel Extraction Kit (Metabion, Germany) and then standard curves were generated based on quantified PCR products with a series of 1:10 dilutions ($R^2 \geq 0.99$ for each gene). All the samples and standards were analyzed in duplicate and several negative controls were included. Amplification efficiencies were calculated as $E=[10^{(-1/slope)} -1]*100$, with the following results: 16S: 97-102%; bacterial *amoA* 88-93%; archeal *amoA* 87-93%; *nirK* 97-99%; *nosZ* 86-90%; *nirS* 82-84% *nifH* 87-93% and *pmoA* 87-90%; these values were consistent with those reported in analogous studies (Töwe el al., 2010; Harter et al., 2014). The abundance of genes was expressed in copy numbers g dw soil-1 according Behrens et al. (2008).

2.6 Greenhouse gas exchange rates

Four periods of exchange rates of carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) were measured in the mesocosms. The temperature and precipitation during the experimental period (**Figure 1**) were consistent with the predominant Mediterranean climate of the area, which corresponds to a warm temperate climate with dry and hot summers or C_{sa} in the Köppen–Geiger climate classification system (Kottek et al., 2006). Within each sampling period, measurements were carried out over a 2-4 day period, once

to twice per day (herein referred as measurement events) between 9:00 and 17:00 h. The gas exchange measurement system consisted of opaque static PVC chambers (60 cm height, enough to allow measurements with grown vegetation, and an internal diameter of 25 cm) connected to a photoacoustic field gas-monitor or PAS (INNOVA 1412, LumaSense Technologies, Denmark) with a multiplexor system allowing twelve simultaneous or sequential measurements. The chambers were placed in the soil by fitting them to PVC rings (7 cm height, 25 cm inner diameter) inserted in the soil since the barley sowing to ensure reasonable sealing of the system and to limit soil disturbance during measurements. The limitations regarding the use of chambers (e.g. discontinuity of measurements, lack of spatial integration, system disturbance) have been discussed widely (de Klein and Harvey, 2012; Flechard et al., 2007; Hutchinson and Livingston, 2002; Rochette and Eriksen-Hamel, 2008; Schrier-Uijl et al., 2009; etc.). All of these limitations are taken into account when interpreting the results but with a limited impact on our conclusions since providing absolute gas balance values for the studied system is not in the scope of this study. Furthermore, it was not an aim of the study to describe GHG annual fluxes dynamics but rather to check differences between biochar treatments at environmentally contrasted moments of the year (e.g. colder, wetter winter versus hotter, drier summer conditions.). The chambers were connected to the gas monitoring equipment with Teflon tubing (2 mm internal diameter). The surface sampled area corresponded to 0.05 m^2 and the chamber volume to 0.03 m^3. The nominal detection limits of the gases are: 3.4, 0.02 and 0.2 ppm for CO_2, N_2O, and CH_4, respectively. The PAS was calibrated prior to the field campaigns by the equipment distributor (Moody et al., 2008). The analyzer was used in the cross-interference and the water-interference modes (for more details on PAS modus-operandi and comparability see Iqbal et al., 2013). Each measurement lasted 20-30 min. The accumulated gas concentrations were measured at regular time intervals (c. every 1-5 min) and those values were used to calculate the gas exchange rates. Since gas concentrations changed linearly with time in the chambers, gas exchange rate values were obtained from the slope of the linear fit to the concentration values (Jones, 1992). Data were submitted to a quality assessment of drifts associated with warm-up process and/or

291 water interference, as recommended by Iqbal et al. (2013). Accordingly, 7% of the CH_4, and
292 1.5% of the N_2O of the exchange rates calculated were not included into the analyses.
293 Positive rate values indicate emission and negative rates denote uptake in soil.
294
295 *2.7 Data analysis*
296 The effect of biochar amendment rates on gas exchange rates, soil chemistry, and biological
297 properties was assessed by generalized mixed-effects models (GLMM) using the "nlme"
298 package by Pinheiro et al. (2016) of R software (R Core Team, 2013). For each gas (N_2O,
299 CH_4 and CO_2) and each rate of application of biochar (LR and HR, corresponding to 5 or 30
300 t ha^{-1} respectively) we carried out a separate GLMM using the application of biochar
301 (amended versus unamended) and sampling (fall 2011, and winter, spring and summer
302 2012) as fixed factors and the mesocosm identity (from 1 to 24, n=8 for each dose of
303 application) and the measurement events (each of the 2-4 consecutive days of
304 measurements per sampling / or that corresponds to a series or batch of measurements) as
305 random factors. We preferred to analyze each rate of application separately because they
306 represent different application scenarios and therefore show different and even opposed
307 responses. Similarly, for the physicochemical and biological measurements, mixed-effects
308 models (GLMM) were also carried out considering the biochar addition or not and
309 samplings as fixed factors, and mesocosm identity as a random factor (see detailed models
310 in **Tables S1**, **S2**, and **S3**). For each mixed model, possible fixed factor interactions
311 structures were checked selecting the structure of the model with the lowest AIC (as
312 explained in Zuur et al., 2010). The model acceptance was based on the graphical
313 diagnostics of residuals to check their normality (QQ-plot and histogram), its homogeneity
314 (residuals *versus* fitted values), and the model fit (fitted values *versus* observed values).
315 Before the construction of the models, data exploration was conducted following Zuur et al.
316 (2010) recommendations, in order to check for outliers, collinearity and explanatory
317 variables relationships. When necessary, variables were transformed by using logarithm
318 (natural or log10) to avoid problems of extreme values or heteroscedasticity (see **Tables S2**,
319 **S3** and **S4)**.

320

321 **3. RESULTS**

322 ***3.1. Effects on soil physicochemical properties***

323 Considering the biochar effects along all of the experimental period (main effects), K^+
324 showed a significant increase (**Figure 2**), up to 25%, in the HR treatment compared to the
325 unamended mesocosms (t=2.89, p=0.009, **Table S1**). Additionally, in HR-mesocosms,
326 higher Mg^{2+} soil concentrations were found with respect to unamended ones (t=2.08,
327 p=0.05). Also, soil moisture content was significantly higher in HR-biochar amended plots
328 over the whole experiment (t=2.29, p=0.0008; **Table S2**), with a mean increase of around
329 15% compared to the unamended soil (**Figure 2**).
330 The interactions between temporality and treatment showed that in the summer sampling,
331 a lower soil moisture was predicted in the HR-biochar mesocosms by the GLMM compared
332 to unamended soil (t=-2.74, p=0.009, **Table S2**), agreeing with the greater water losses
333 observed in this treatment (**Figure 2**). In addition, a significant interaction indicated higher
334 summer depletion of SO_4^{2-} and K^+ in the HR treatment compared to unamended soil (t=-
335 2.40, p=0.02 and t=-2.56, p=0.03 respectively; see **Figure 2** and **Table S2**).
336 For the other sampling times, a significantly higher NO_2^- depletion was observed in the
337 spring sampling in the HR versus controls (t=-3.33, p=0.02, **Table S2**, and **Figure 2**),
338 together with a marginally significant Ca^{2+} enrichment in HR compared to unamended soil
339 (t=1.72, p=0.09, **Table S2** and **Figure S1**). Meanwhile, in the winter sampling, marginally
340 significant depletion in NO_2^- (t=-2.21, p=0.05; **Table S2**, and Figure 2) and NH_4^+ (t=-0.85,
341 p=0.06; **Table S2**, and **Figure 2**) was found in the HR treatment, together with significant
342 higher NO_3^-/NH_4^+ ratios and $NO_2^-+NO_3^-/NH_4^+$ relationships in HR-applied soils (t=2.47,
343 p=0.02; and t=2.31, p=0.03 respectively, **Table S2**), as well as a decreased K^+ soil content
344 compared to unamended soil (t=-2.80, p=0.01, **Table S2**, and **Figure 2**).
345 In the LR-biochar mesoscosms, during the whole experimental period (main effects), we
346 found a significant enhanced depletion of Na^+ content compared to unamended soil (t=-
347 2.05, p=0.05, **Table S2**; **Figure S1**). However, in the spring the LR-applied mesoscosms
348 presented Na^+ enrichment in comparison to unamended soil (t=2.2, p=0.03, **Table S2**,

Figure S1), as also observed for Ca^{2+} (t=1.95, p=0.05, **Table S2**, **Figure S1**) and Cl^-, although in the last case marginally (t=1.77, p=0.08; see **Table S2**, **Figure S1**). In the winter sampling, HPO_3^- soil concentrations were enriched in LR compared to unamended microcosms (t=2.08, p=0.04, **Table S2**, **Figure S1**), in a trend that was found in the HR-mesocosms although it was not significant (t=1.64, p=0.1, **Table S2**).

3.2. Effects on soil biological properties

Significantly higher values of basal respiration (BR, **Figure 2**) were globally found in HR-biochar-amended mesocosms along the experimental period (t=2.48, p=0.02; **Table S3**) but not for microbial biomass carbon (MBC) or DOC (dissolved organic carbon, see **Figure S1**). Similarly, the bacterial abundance did not vary significantly when measured as 16S rRNA gene copy numbers (**Figure 3, Figure S2**), although for the winter sampling, a marginally significant increase in HR-applied soils was detected (t=1.7, p=0.09; **Table S3**). Regarding the functional gene abundance (**Figure 3**), no differences were found for high biochar addition rate in *nirK*, *nosZ*, *nifH*, bacterial *amoA* (AOB) and archeal *amoA* gene (AOA) copies number, with the exception of the marginally significant increase in *nirS*/*nirK* ratio (t=1.76, p=0.09; **Table S3**). Similarly, no significant interactions were found between sampling and HR application rate, with the exception of the marginally significant temporal decrease in the AOA/AOB ratio (**Figure S2**) at the winter sampling, t=-1.83, p=0.07; **Table S3**).

However, regarding LR addition rate scenario (see **Figure 3**), our results denoted a global significant increase in the AOA/AOB ratio (t=2.06, p=0.05; **Table S3**), and a decrease in *nosZ*/(AOA+AOB) (t=-2.47, p=0.02; **Table S3**) and *nosZ*/AOA ratios (t=-2.13, p=0.05; **Table S3**). Regarding the interactions between LR-treatment and sampling, we demonstrated a lower *nosZ*/(*nirS*+*nirK*) ratio in the summer sampling (t=-2.24, p=0.03; **Table S3**). Also, although marginally, there was an increase in the spring sampling in the *nosZ*/(AOA+AOB) ratio (t=1.71, p=0.09; **Table S3**) and a decrease in the *nosZ*/AOA (t=-2.47, p=0.02; **Table S3**) and AOA/AOB ratios (t=-1.88, p=0.06; **Table S3**) in the winter sampling. In this last case, the same trend was observed in the HR treatment although was marginally significant (t=-

1.83, p=0.07; **Table S3**). Regarding the metabolic quotient (qCO_2), a marginally significant interaction suggests a decrease in the spring sampling (t=-1.82, p=0.07; **Table S3**). For both the HR and LR treatments, *pmoA* gene copies did not show any significant effects or interactions with sampling time (**Figure 3**).

3.3. Effects on GHG exchange rates

Although no global effects of biochar addition rates were found for any of the measured GHG along the samplings, significant interactions with sampling time were found for N_2O and CH_4. Namely, in the summer sampling, the N_2O emissions showed contrasting responses in the different biochar treatments (**Figure 4**). In the HR treatment, a significant and strong reduction was observed (t=-2.34, p=0.02, **Table S4**), with negative rates (-0.07 ± 0.08 N_2O mg m^{-2} h^{-1}, n=29) compared to the positive rates observed in unamended mesocosms (0.29 ± 0.13 N_2O mg m^{-2} h^{-1}, n=30). On the contrary, positive rates were observed in the LR treatment (0.32 ± 0.12 N_2O mg m^{-2} h^{-1}, n =32), as observed in the controls, without significant differences.

Regarding CH_4 emissions (**Figure 4**), a strong increase was observed in summer in the biochar-amended mesocosms, with higher rates in HR (8.88 ± 2.57 mg m^{-2} h^{-1}, n=28) compared to the unamended mesocosms (1.89 ± 2.11 mg m^{-2} h^{-1}, n=29) which was significant (t=2.25, p=0.03, **Table S4**). In case a more extensively characterization of GHG would confirm these patterns, the increase in CH_4 might offset the N_2O decrease related with the HR biochar application in the soil. Again, the emissions in the LR treatment were not significant (2.35 ± 2.45 mg m^{-2} h^{-1}, n=31) compared to controls.

Soil CO_2 emissions were not significantly affected by the biochar treatments at any of the application rates or samplings (**Figure 4**, **Table S4**). Average CO_2 exchanges rates ranged between 0.37 and 0.27 CO_2 g m^{-2} h^{-1} depending on the sampling, with 0.27 ± 0.009 for the spring (n=106), and 0.37 ± 0.02 for the summer and winter sampling (n=96 and n=48, respectively), and 0.38 ± 0.09 CO_2 g m^{-2} h^{-1} in fall (n=83).

406 ## 4. DISCUSSION

407 ### *4.1. Soil physicochemical properties: water and ionic content shifts at the high biochar*
408 ### *application rate scenario*

409 Within 8 to 21 months after application, higher water contents and soluble K^+ and Mg^{2+}
410 concentrations were found along the period of study under our high biochar application
411 rate scenario (30 t ha^{-1}), but not in the lower rate scenario (5 t ha^{-1}). This agrees with similar
412 studies that have reported enhanced soil water retention (Karhu et al., 2011; Novak and
413 Watts, 2013; Saarnio et al., 2013) and K^+ or Mg^{2+} nutrient contents (Angst and Sohi, 2013;
414 Lehmann et al., 2003; Novak et al., 2009). These findings could be related to the expected
415 enhancement of cation retention capacity of biochars or cation releases derived from
416 biochar or its mineralization of the labile biochar fraction, and biochar porosity in specific
417 case of moisture. Regarding any effects on soil pH, such as have been reported in some
418 studies (Jones et al., 2012; Major et al., 2010; Novak et al., 2009), and despite the high pH
419 of this gasification biochar (around 11), we failed to find any variation plausibly due to the
420 alkaline nature of the soil in this study (over 8). No effects were found for either of the
421 biochar rates tested for dissolved organic C (DOC), in contrast to other studies that found
422 increased values shortly after the application (Angst and Sohi, 2013; Lehmann et al., 2003;
423 Novak et al., 2009).

424 When variation within samplings were assessed, some significant trends (decreases) were
425 observed for NO_2^- (spring and winter sampling) and NH_4^+ contents (winter sampling), but
426 not for NO_3^-, although a non-significant trend to lower concentrations is suggested from
427 our results. However, the NO_2^-+NO_3^-/NH_4^+ ratio increased in the winter sampling of the
428 HR treatment. In the summer sampling, SO_4^{2-}, K^+, and water content experienced declines
429 in the HR biochar-treated soils (**Figure 2**). For the other samplings, the only remarkable
430 effect is the increase in Ca^{2+} and Na^+ under HR of application.

431 The literature evidence suggests that biochar aging can increase its cation adsorption
432 capacity (Hale et al., 2012; Jones et al., 2011), as well as adsorption of nitrogen compounds
433 (Adams et al., 1988; Seredych and Bandosz, 2007; Uchimiya et al., 2012; Wang et al., 2012).
434 This might explain the higher contents of Mg^{2+}, Ca^{2+}, Na^+, K^+ in soils amended with HR,

435 but not the nitrogen forms, for which we only found reductions in the nitrite and
436 ammonium contents in some samplings, or no effects in the specific case of nitrates. The
437 importance of cation adsorption in our study is probably small due to the time required for
438 biochar aging (Mia et al., 2017) and the initial low CEC of the gasification biochar of this
439 study (Pérez-Herrero, 2013). This suggests that the Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} enrichment in HR
440 plots results from direct release from biochar or its initial mineralization (see biochar
441 characterization in Marks et al., 2014).
442
443 *4.2. Soil microorganisms: increased activity without effects on abundance and functional*
444 *groups at the high application scenario*
445 Microbial abundance was not significantly higher in any of the biochar-amended
446 mesocosms, marginally at most, contrasting with other studies that have reported higher
447 microbial biomass (Ameloot et al., 2013b; Luo et al., 2013; Singh and Cowie, 2014), as
448 expected for biochar that could act as a more suitable habitat and a refuge from
449 microbivores (Lehmann et al., 2011; Thies et al., 2015). Concerning microbial activity,
450 measured as basal respiration (BR), and assessed under laboratory conditions, it was
451 significantly greater in soils where biochar had been applied, agreeing with other published
452 studies (Case et al., 2012; Saarnio et al., 2013), although we have only found significant
453 effects for the HR treatment, and uncoupled to any change in biomass or metabolic quotient
454 (qCO_2). The decrease in qCO_2 observed in other biochar studies (Jin, 2010), suggesting
455 higher carbon use efficiency of the microbial communities that have been interpreted as
456 improved soil quality (Franchini et al., 2007) due to a decreased energy spending on the
457 community (Anderson, 1994), was not observed in our study, with the exception of the
458 marginally significant effect in the spring sampling. Hence, we could not demonstrate the
459 expected biochar soil improvement for microorganisms, in agreement with Quilliam et al.
460 (2013). The sustained increased respiration in samples from HR biochar plots in all the
461 samplings (with no higher biomass levels or qCO_2 compared to controls) could suggest a
462 higher availability of labile carbon in this treatment and/or the result of community shifts.
463 Such a respiration increase was not observed under field conditions probably due to the

464 myriad of processes affecting CO_2 emissions in real scenarios. The lack of effects under
465 field conditions has been widely reported in the biochar literature, using different biochar
466 feedstocks (made from cereal remains, wood, shells, or manure), amendment rates (4–200
467 tons/ha), soil types (from silty clay to sandy), environmental conditions (from boreal to
468 subtropical climate), or experiment durations (from 25 days to 2.9 years) (Ameloot et al.,
469 2013b; Castaldi et al., 2011; Kammann et al., 2011; Karhu et al., 2011; Mukome et al.,
470 2013; Scheer et al., 2011; Singh et al., 2010; Spokas and Reicosky, 2009; Zhang et al.,
471 2012a).
472 Several studies have documented that biochar induces shifts in the microbial community
473 composition (Anderson et al., 2011; Ducey et al., 2013; Khodadad et al., 2011; Steinbeiss et
474 al., 2009). However, in our study, we could not demonstrate changes in the total and
475 relative abundance of N and C-cycle functional microbial groups (as total copies per gene,
476 and as percent of the 16S copies, respectively). These results agree with other studies that
477 failed to find effects on nitrification and denitrification functional genes abundance (AOA,
478 nirS, nirK, and nosZ) (Anderson et al., 2014; Dicke et al., 2015; Ducey et al., 2013; Imparato
479 et al., 2017; Prommer et al., 2014; Song et al., 2014; Xu et al., 2014). However, this
480 contrasts with Wang et al. (2013), who reported biochar-induced increases in *nirS and nosZ*
481 (denitrifiers) gene copy numbers together with a decrease in *nirK* copies, and with Harter
482 et al. (2014) who also reported higher *nosZ* copy numbers. This might partly explain the
483 lack of biochar effects on the soluble NO_2+NO_3 contents measured, and might agree with
484 the study by Castaldi et al. (2011), also carried out under Mediterranean conditions, who
485 reported no significant changes in nitrification in a field experiment.
486 However, we found changes in some functional group ratios under the LR treatment.
487 Namely, less denitrifiers per ammonia oxidizers were globally observed (lower
488 *nosZ*(AOA+AOB) and the *nosZ*AOA ratios). Similarly, less denitrifiers per nitrate
489 reducers were also found (*nosZ*(*nirS*+*nirK*)), but only for the summer samplings. In the
490 spring sampling, an increase in denitrifiers per Archaea ammonia oxidizers (*nosZ*AOA
491 ratios) was found. The relative decrease in denitrifiers may potentially lower the N_2O
492 removal capacity in LR plots (*nosZ* is associated with the conversion of N_2O to N_2), but this

493 was not associated with increased N_2O emissions. Regarding the HR treatment, where
494 significant N_2O mitigation was observed, no significant variation in functional ratios were
495 observed.
496
497 ***4.3. GHG exchange rates: reduced N_2O and enhanced CH_4 emissions in summer at the high***
498 ***application scenario***
499 In our study, the higher N_2O and CH_4 emission rates were observed in the summer
500 sampling, after barley harvest, at least partly associated with the higher temperatures
501 (>35ºC at 5-7 cm depth), while similarly lower rates were found in the other samplings.
502 Taking into consideration the biochar treatments, significant impacts on the N_2O and CH_4
503 emissions were observed, but not on the CO_2 exchange rates, ranging from mitigation to
504 emission enhancements.
505 In the summer sampling, N_2O emissions were significantly lower (and negative) at the HR
506 treatment compared to the positive emission rates observed in unamended soil and the LR
507 treatment, in contrast to the general negative rates observed for all the other samplings
508 and treatments. The N_2O mitigation by biochar addition in our study agrees with other
509 studies (Castaldi et al., 2011; Cayuela et al., 2013; Nelissen et al., 2014) which have
510 demonstrated this effect in biochars with low nitrogen content like the one in our study
511 (Cayuela et al., 2013), as expected for a wood biochar compared to animal manures or food
512 wastes (Spokas, 2013). This effect is coupled to an enhancement in the release of CH_4, with
513 the lack of changes in CO_2 emission rates.
514 Our results are of interest as they describe N_2O and CH_4 emissions (i) under field
515 conditions; ii) at the medium-term; and (iii) with a temporal sampling able to detect effects
516 restricted to particular environmental conditions (summer in our study). Our approach
517 contrasts with most of the available published studies, mostly under laboratory conditions
518 and assessing GHG effects shortly after the field biochar application, with incubation times
519 below 1 year (e.g., Bruun et al., 2011; Cayuela et al., 2010; Spokas, 2013). Only considering
520 short-term effects might bias the estimation of biochar on emission rates. Due to biochar
521 aging in soil, the time passed since the biochar application could strongly affect soil

processes and lead to unexpected outcomes that need to be assessed experimentally. As an example, Nelissen et al. (2015), only observed short-term changes in aerobic nitrogen transformations in the field shortly after the application but not after 1 year. The experimental design of our study, assessing GHG effects 8 to 21 months following biochar application might overcome such bias, and highlight the need to consider seasonality in experimental designs to be able to enhance the precision of the climate change mitigation capacity of biochar. Moreover, the contrasting results obtained in low and high application scenarios in this study using the same biochar and soil, reveal that mitigation potential is highly rate-dependent.

4.4. N_2O emission mitigation: net consumption in biochar anaerobic microsites

The mechanisms of biochar interactions with N_2O have rarely been evaluated. and the relative change (decrease, increase or no change) in emissions is a result of the net effect of several abiotic and biotic mechanisms operating concurrently (Van Zwieten et al., 2015). Several mechanisms have been proposed to explain N_2O emissions decrease after biochar addition. Firstly, N_2O emissions could be decreased compared to the unamended soil due to an increase in soil pH by the addition of biochars, which favors N gaseous emissions but decreases the ratio N_2O:N_2 (Simek and Cooper, 2002). This mechanism is not plausible in our study, as our soil pH was already alkaline, and accordingly unaffected by biochar addition.

A second mechanism might be the N adsorption capacity of biochar, increasing with aging (Singh et al., 2010), consequently reducing the availability of mineral N used by N-cycle bacteria. The adsorption could be through the direct retention in biochar as NH_4^+, or in the specific case of NO_3^-, by bridging through divalent or trivalent cations associated with biochar surface (Mizuta et al., 2004; Mukherjee et al., 2011; Tsukagoshi et al., 2010), which should end up slowing down N_2O emissions (Karhu et al., 2011; Kettunen and Saarnio, 2013). This mechanism is also unlikely in our study since the soluble (bioavailable) NH_4^+, NO_3^- and NO_2^- contents did not decrease in the HR treatment in summer, when N_2O rates decreased significantly.

A third mechanism could be direct N_2O adsorption promoted by biochar's high specific surface area (Peng et al., 2009). Although we cannot discard this possibility, we consider this mechanism unlikely in our experiment since higher N_2O adsorption should be present along the different samplings, which is not the case.

A fourth mechanism might be the shifts in microbial community structure or activity associated with the modification of the soil habitat by biochar (Lehmann et al., 2011). Specifically, biochar application can provide labile carbon and inorganic substances, or even toxic compounds, can promote the retention of inorganic and organic compounds on its highly reactive surface, and provide abundant pores potentially acting as refuge or as more suitable microhabitats, hence potentially affecting microbial composition. Those shifts can in turn lead to changes in microbial functions such as the N-cycle (Clough et al., 2013). In our study, we failed to find any changes in total and relative abundance of N-cycle functional groups, with the exception of some functional group ratios uncoupled with the observed emissions. This was in contrast to other studies finding shifts (Ducey et al., 2013; Harter et al., 2014; van Zwieten et al. 2014), but in agreement with others (Anderson et al., 2014; Dicke et al., 2015). However, since significant impacts on N_2O were observed, and no other mechanism seems to be applicable to our results, it is plausible that changes in microbial activity (measured as transcripts) with no changes in the abundance (measured as genes) could explain this disagreement. As an example of that, Xu et al. (2014), failed to find any increase in AOA gene copy numbers, but did in the numbers of the corresponding transcript, the latter explaining the increase in nitrification rates they observed. All that said, we hypothesized that the key for the decreased N_2O emissions in our study might result from the biochar porous structure that provides abundant microsites which can become anoxic when water saturated (Hagemann et al., 2016) or when microbial activity inside those pores is so intense that exhaust oxygen (Harter et al., 2014; van Zwieten et. al., 2009). This is something that promotes complete denitrification (from NO_3^- to N_2O and then completely reduced to N_2) using organic carbon as electron donor, hence globally reducing N_2O emission rates (Hagemann et al., 2016). The summer sampling positive N_2O emission rates in controls and LR scenario coupled to the negative

N_2O emissions in the HR scenario seem to support this hypothesis: the combination of maximum microbial activity at the high summer temperatures in all the treatments (**Figure 5**) with the HR scenario, with more biochar content and potentially more anoxic microsites present, could explain the complete denitrification to N_2 by total N_2O consumption. This highlights the need for N_2 measurements coupled to N_2O assessments for a better understanding of the underlying mechanism for N_2O mitigation.

4.5 CH_4 emissions enhancement: net release from biochar anaerobic microsites

Many studies, using different types of soils and biochars, have generally demonstrated that biochar addition does not affect CH_4 fluxes in aerated (non-saturated) soils (Aguilar-Chávez et al., 2012; Angst et al., 2014; Scheer et al., 2011; Spokas and Reicosky, 2009; Troy et al., 2013; Wang et al., 2012). However, in some cases biochar addition can increase (Karhu et al., 2011; Yu et al., 2013; Zhang et al., 2012b) or decrease net CH_4 oxidation (Spokas et al., 2009; Spokas and Reicosky, 2009; Zhang et al., 2012b).

CH_4 is produced under anoxic conditions and this is when there is the lowest CH_4 consumption (oxidation) (Bodelier, 2011; Mosier et al., 1998), so one might expect minimum emissions in summer, with the lower moisture contents. It has been suggested that biochar improves soil aeration, through the increased macroporosity, especially in wood-derived biochars (Downie et al., 2009), important in maintaining aerobic conditions in soil (Van Zwieten et al., 2009). Thus, biochar could decrease anoxic conditions in soils, which could potentially decrease CH_4 production and/or increase CH_4 oxidation (Van Zwieten et al., 2009). However, our study showed maximum CH_4 emissions in summer and hence support the idea that anoxic conditions in aerated and drained soils are present in microsites with anoxic conditions (Hagemann et al., 2016), in agreement with the mechanism proposed for N_2O. Our results disagree with those of Karhu et al. (2011), who studied plots in southern Finland and concluded that the highest aeration in biochar-added soils caused decreased CH_4 emission through enhanced CH_4 oxidation. This disagreement might be the result of different main mechanisms explaining net CH_4 emissions and probably related to the higher soil temperatures in our plots, under Mediterranean climate (26-53ºC, with soil

609 moisture around 8-15%), compared to that in Karhu et al. (2011), under Boreal climate (17-
610 25ºC, with soil moisture of 10-16%). The higher temperatures under the Mediterranean
611 climate, under similar water contents might promote higher bacterial activity and oxygen
612 consumption within biochar micropores, and therefore promote anoxic conditions in
613 microsites that allow net CH_4 emissions. This microsite biologically-induced anaerobiosis
614 hypothesis could be also supported by the significant reductions in SO_4^{2-} in summer plots
615 treated with biochar in our experiment, since sulphate reducers also operate under these
616 conditions (Segers, 1998). The strong decrease in SO_4^{2-} might agree with its preferential
617 use as electron acceptor (and conversion to HS^-) according to its higher potential redox in
618 comparison to the CO_2 used as electron acceptor for CH_4 production, which is a
619 thermodynamically less efficient process (Conrad, 1989; Lovley and Phillips, 1987;
620 Oremland, 1988).
621 However, other mechanisms might concur in the trend observed in the summer sampling
622 and associated with biochar application, such as: i) higher pH, which favours methanogens
623 over methanotrophs, the latter being strict aerobics, and thus favouring CH_4 production,
624 or ii) higher DOC contents which could contribute to more biologically-induced anoxic
625 conditions within biochar pores, and which is consistent with our measurements. However,
626 in our study we failed to find significant differences in the HR plots on any of these
627 parameters (**Figure S1**). Similarly, and as found for N_2O, bulk soil community shifts are
628 not plausible, at least in methanotrophs when measured as *pmoA* abundance (**Figure 3**).
629

630 **CONCLUSIONS**

631 Our study demonstrates a biochar impact on N_2O and CH_4 emissions within the two first
632 years following the application of a pine gasification biochar, under Mediterranean
633 conditions, when applied at relatively high rates (30 t ha^{-1}), but not at low rates (5 t ha^{-1}),
634 though restricted to the summer sampling. Such effects, unrelated to pH, bioavailable
635 nitrogen status, and microbial community shifts in bulk soil, were possibly related to the
636 porous structure of our wood biochar, which was able to provide microsites with clearly
637 different environmental conditions in comparison to bulk soil. We hypothesized that

638 despite the relatively low moisture contents, the higher summer temperatures promoted
639 biologically-induced anoxic conditions in biochar pores, where NO_3^- is totally denitrified to
640 N_2 thus leading to negative N_2O exchange rates, and where CH_4 could have net release
641 rates.
642 Our results highlight the need for an accurate assessment of biochar GHG mitigation
643 capacity, and hence considering the simultaneous assessment of the main GHG (N_2O, CH_4,
644 and CO_2), the seasonality of the emissions to fully cover the range of climatic conditions of
645 the area of study, and to assess medium- to long-term effects under field conditions to
646 include aging effects.
647
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1120

FIGURE CAPTIONS

Figure 1. Mean daily temperature (empty dots), maximum daily temperature (filled dots), and 24-hour accumulated precipitation during the study period in Cerdanyola del Vallès, the closest weather station (c.a 4 km from experimental set up). Data has been provided by the Meteorological Service of Catalonia (XEMEC). The arrows in the top indicate the sampling dates. The figure contains also culture information: 2 periods of fertilization (black dotes) and the period of crop development from seeding to harvesting (vertical lines).

Figure 2. Mean soil water-soluble ions, moisture, and basal respiration per sampling and biochar treatments. Only those with significant changes during the experimental period are shown (see Table S2 for the GLMM probability values).

Figure 3. Heatmap of bacterial gene abundance (log_{10} of the copies number) during the experiment in the different biochar addition treatments.

Figure 4. Mean emission rates of N_2O, CH_4 and CO_2 per sampling and biochar treatment.

Figure 5. N_2O and CH_4 emission relationship with soil temperature (at 5-7 cm depth) in the control and the highest application rate treatments.

Figure 1

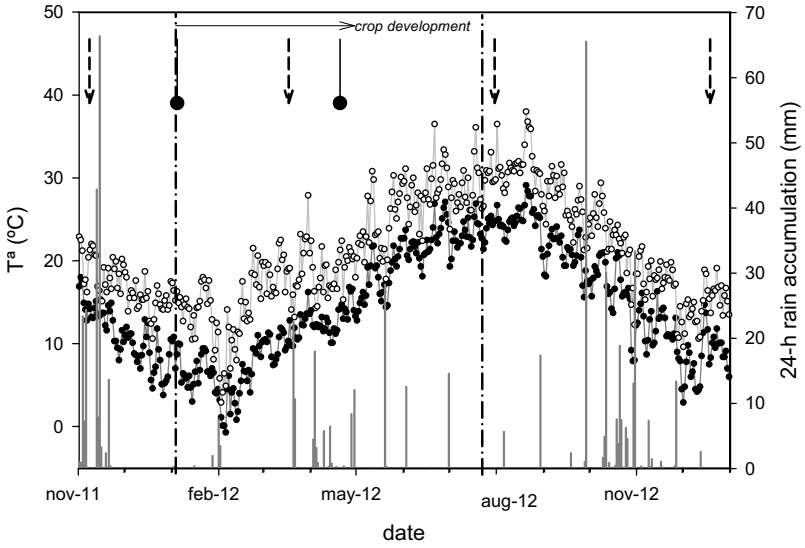

crop development
Tª (ºC)
24-h rain accumulation (mm)
nov-11
feb-12
may-12
aug-12
nov-12
date

Figure 2

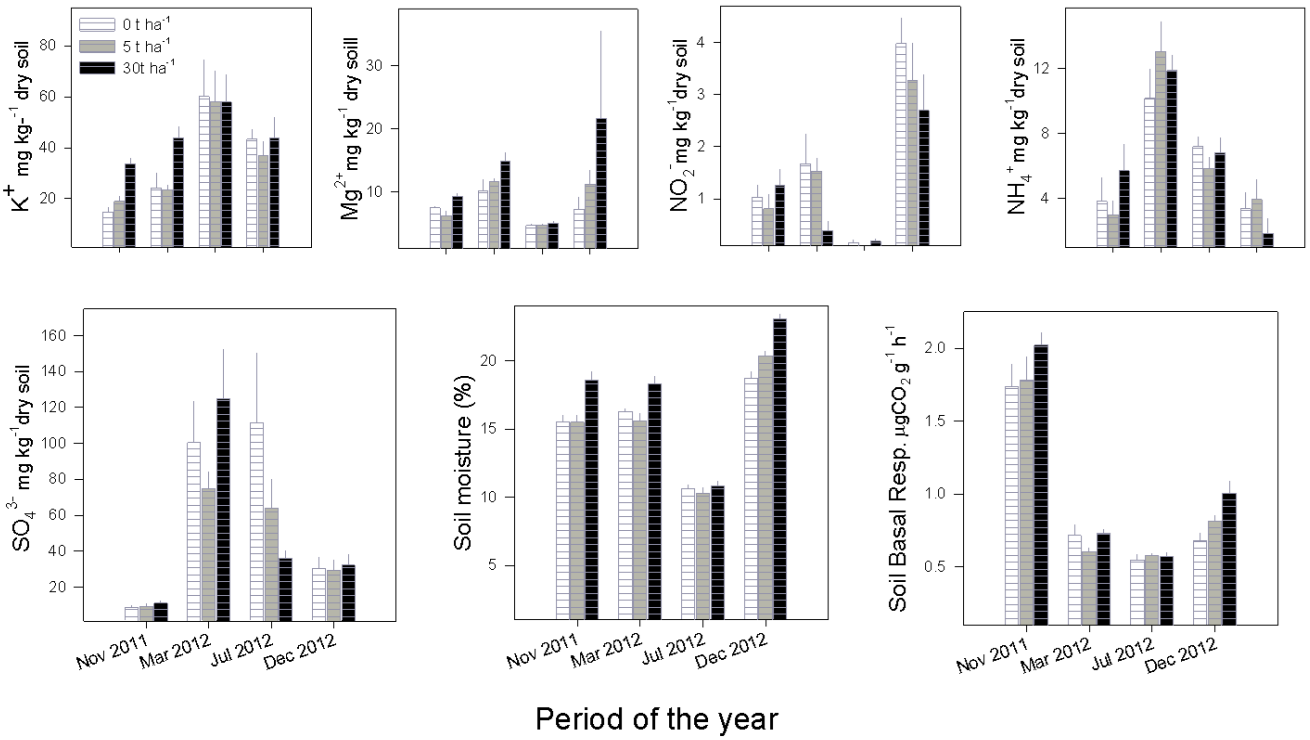
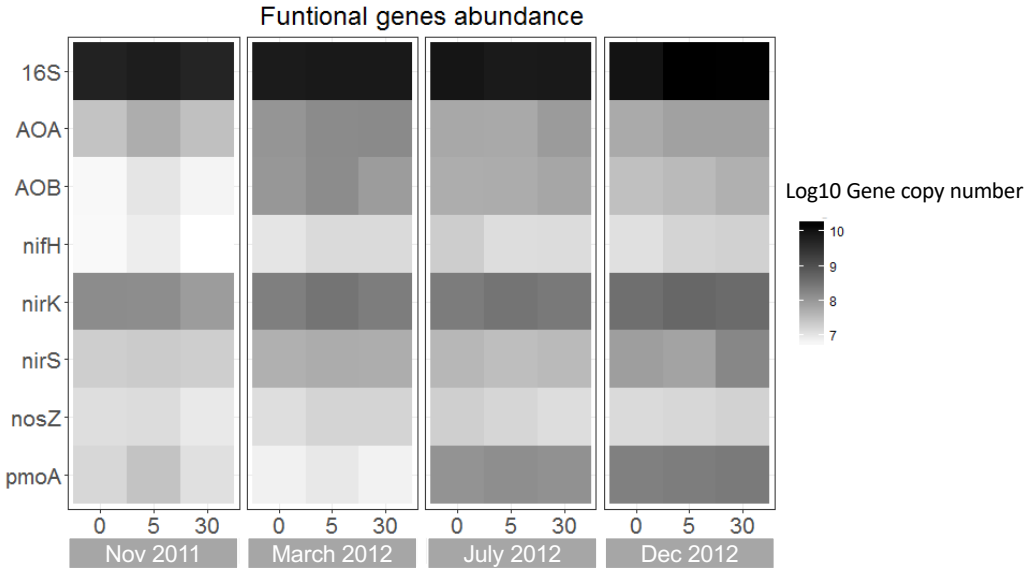
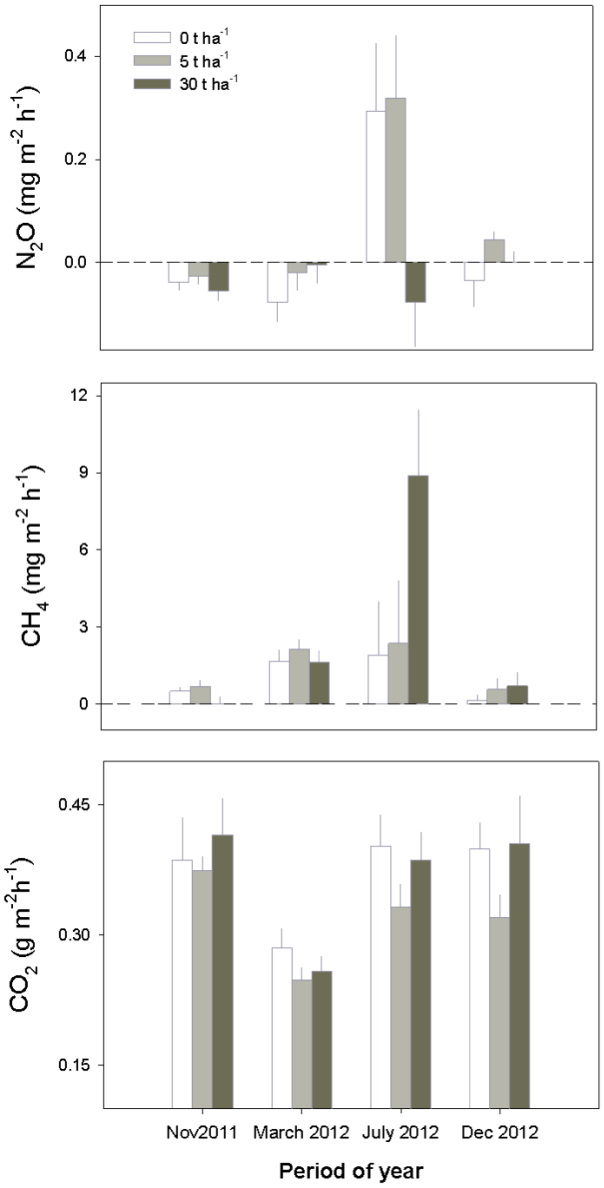

0 t ha-1
5 t ha-1
30t ha-1
K+ mg kg-1 dry soil
Mg2+ mg kg-1 dry soill
NO2- mg kg-1 dry soil
NH4+ mg kg-1 dry soil
SO4 3- mg kg-1 dry soil
Soil moisture (%)
Soil Basal Resp. µgCO2 g-1 h-1
Nov 2011
Mar 2012
Jul 2012
Dec 2012
Period of the year

Figure 3


Funtional genes abundance
16S
AOA
AOB
nifH
nirK
nirS
nosZ
pmoA
0 5 30
Nov 2011
March 2012
July 2012
Dec 2012
Log10 Gene copy number
10
9
8
7

1 Figure 4



2

3

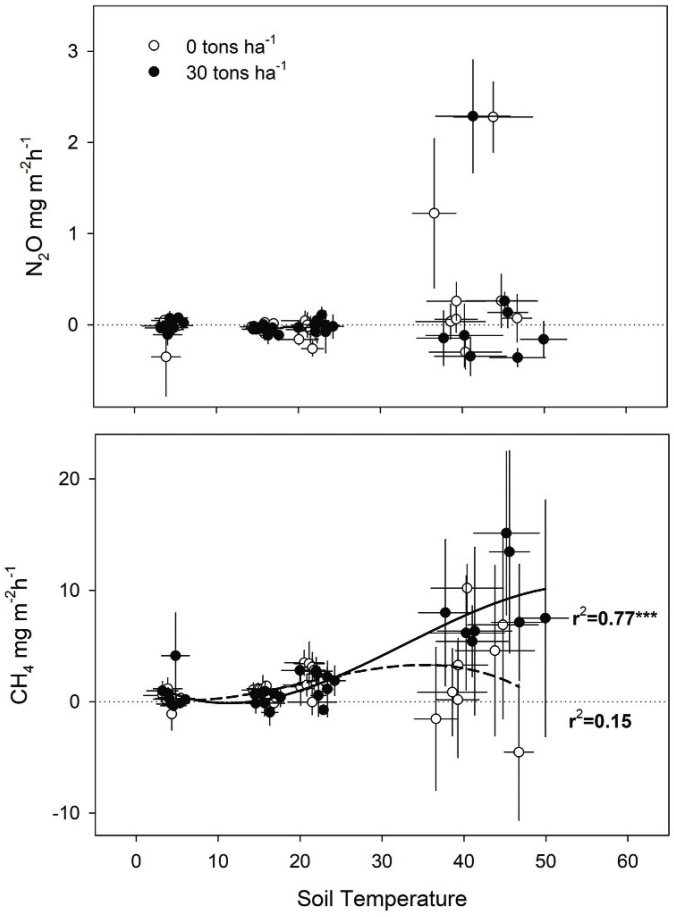
4

5

6

7

Figure 5


0 tons ha-1
30 tons ha-1
N2O mg m-2h-1
CH4 mg m-2h-1
r2=0.77***
r2=0.15
Soil Temperature

Supplementary Materials

Table S1. Primers and thermal profiles used for real-time PCR quantification of the different target genes.

Target gene	Primers	Thermal profile	Number of cycles	Reference
16S rRNA	EUB338 EUB518	95°C- 45 s / 53°C- 45 s / 72°C,-45 s	40	Fierer et al., 2005
nifH	nifHF nifHR	95 °C – 45 s/ 55 °C – 45 s/ 72 °C – 45 s	40	Rosch et al; 2002
amoA AOA	amo19F CrenamoA16r48x	95°C, 45 s / 55°C, 45 s / 72°C, 45 s	40	Leininger et al., 2006 Schauss et al., 2009
amoA AOB	amoA1F amoA2R	95 °C – 45 s/ 60 °C – 45 s/ 72 °C – 45 s	40	Francis et al; 2005
nirK	nirK876C nirK1040	95 °C – 15 s/63 °C – 30 s/72 °C – 30 s 95 °C – 15 s/58 °C – 30 s/72 °C – 30 s	6[a] 40	Henry et al; 2004
nosZ	nosZ2F nosZ2R	95 °C – 15 s/65 °C – 30 s/72 °C – 30 s 95 °C – 15 s/60 °C – 30 s/72 °C – 30 s	6[a] 40	Henry et al; 2006
nirS	nirScd3aF nirSR3cd	95 °C –15 s/ 57 °C – 30 s/ 60 °C – 15 s	40	Michotey et al. (2000) Throback et al. (2004)
pmoA	A189 A682	95 °C –30 s/58.5 °C – 30 s/72 °C – 30 s	40	Juottonen et al., 2006

[a] touchdown -1°C for cycle

Table S2. Generalized mixed models (GLMM) evaluating the effect of biochar addition on soil chemical properties (only the models with significant or nearly significant results are shown): a) Linear mixed-effects model fit by REML we carried out in R software (lme4 package) with the formula lme.var.<-lme(var.~HR-biochar, random=~1|Mesocosm), where HR-biochar corresponds to the biochar application rate comparison between 0 versus 30 t ha^{-1}, and season corresponds to each of the sampled periods during the year (fall 2011, springtime 2012, summer 2012 or winter 2012); and b) similarly, in this case linear mixed-effects model with the formula lme.var.<-lme(var.~LD-biochar, random=~1|Mesocosm), where LD-biochar corresponds to the biochar application rate comparison between 0 versus 5 t ha^{-1}. For each model, the transformation method of the response variable is shown together with the number of available observations (n) used for each model construction. Rows in bold highlight statistically significant results ($p<0.05$) regarding biochar amendments.

a. HR- of biochar application

Potassium, ln(x), n=64

Random effects	Variance
Mesocosm	0.26
Residual	0.52

Fixed Effects	Value	Std. error	df	t	p
intercept	2.62	0.21	42	12.70	0.0000
HR-biochar	**0.87**	0.29	14	2.98	**0.0099**
factor (spring)	0.21	0.26	42	0.78	0.44
factor (summer)	1.29	0.26	42	4.94	0.0000
factor (winter)	1.10	0.26	42	4.20	0.0001
HR-biochar*factor(spring)	0.04	0.37	42	0.12	0.90
HR-biochar*factor(summer)	**-0.83**	0.37	42	-2.56	**0.03**
HR-biochar*factor(winter)	**-0.97**	0.37	42	-2.80	**0.01**

Magnesium, ln(x+2), n=64

Random effects	Variance
Mesocosm	0.09
Residual	0.50

Fixed Effects	Value	Std. error	df	t	p
intercept	2.19	0.14	45	15.24	0.0000
HR-biochar	**0.28**	**0.13**	**14**	**2.08**	**0.05**
factor (spring)	0.24	0.18	45	1.37	0.17
factor (summer)	-0.42	0.18	45	-2.36	0.02

factor (winter)	-0.06	0.18	45	-0.36	0.72

Calcium, ln(x), n=64

Random effects	Variance
Mesocosm	0.045
Residual	0.43

Fixed Effects	Value	Std. error	df	t	p
intercept	5.09	0.15	42	33.53	0.0000
HR-biochar	-0.05	0.21	14	-0.22	0.83
factor (spring)	-0.13	0.21	42	-0.62	0.53
factor (summer)	-0.39	0.21	42	-1.86	0.07
factor (winter)	0.96	0.21	42	4.50	0.0001
HR-biochar*factor(spring)	**0.52**	**0.30**	**42**	**1.72**	**0.09**
HR-biochar*factor(summer)	-0.06	0.30	42	-0.21	0.83
HR-biochar*factor(winter)	0.06	0.30	42	0.20	0.84

Nitrite, ln(x+2), n=64

Random effects	Variance
Mesocosm	0.06
Residual	0.27

Fixed Effects	Value	Std. error	df	t	p
intercept	1.08	0.11	66	5.37	0.0000
HR-biochar	0.07	0.006	22	0.88	0.39
factor (spring)	0.14	0.13	66	2.43	0.01
factor (summer)	-0.32	0.13	66	-3.39	0.001
factor (winter)	0.68	0.13	66	6.76	0.0000
HR-biochar*factor(spring)	**-0.44**	**0.008**	**66**	**-3.33**	**0.02**
HR-biochar*factor(summer)	-0.05	0.008	66	-0.46	0.78
HR-biochar*factor(winter)	**-0.37**	**0.008**	**66**	**-2.21**	**0.05**

Ammonium, ln(x+1), n=64

Random effects	Variance
Mesocosm	2.08e-05
Residual	0.64

Fixed Effects	Value	Std. error	df	t	p
intercept	1.26	0.23	42	5.51	0.0000
HR-biochar	0.35	0.32	14	1.09	0.29
factor (spring)	0.94	0.32	42	2.90	0.005
factor (summer)	0.82	0.32	42	2.55	0.01
factor (winter)	0.05	0.32	42	0.15	0.87
HR-biochar*factor(spring)	-0.01	0.45	42	-0.03	0.97

HR-biochar*factor(summer)	-0.42	0.45	42	-0.91	0.36
HR-biochar*factor(winter)	**-0.85**	**0.45**	**42**	**-1.88**	**0.06**

Phosphate, ln(x), n=64

Random effects	Variance
Mesocosm	0.21
Residual	0.42

Fixed Effects	Value	Std. error	df	t	p
intercept	0.83	0.16	42	4.99	0.0000
HR-biochar	-0.003	0.23	14	-0.01	0.99
factor (spring)	0.84	0.21	42	4.05	0.0002
factor (summer)	1.41	0.21	42	6.75	0.0000
factor (winter)	0.82	0.21	42	3.94	0.0003
HR-biochar *factor(spring)	-0.15	0.29	42	-0.51	0.61
HR-biochar*factor(summer)	-0.33	0.29	42	-1.13	0.26
HR-biochar*factor(winter)	0.48	0.29	42	1.64	0.10

Sulphate, ln(x), n=63

Random effects	Variance
Mesocosm	2.3e-05
Residual	0.69

Fixed Effects	Value	Std. error	df	t	p
intercept	1.91	0.24	41	7.82	0.0000
HR-biochar	0.39	0.35	14	1.13	0.28
factor (spring)	2.42	0.35	41	6.99	0.0000
factor (summer)	2.43	0.36	41	6.78	0.0000
factor (winter)	1.22	0.35	41	3.53	0.001
HR-biochar*factor(spring)	-0.04	0.48	41	-0.07	0.94
HR-biochar*factor(summer)	**-1.20**	**0.48**	**41**	**-2.40**	**0.02**
HR-biochar*factor(winter)	-0.16	0.48	41	-0.32	0.74

NO_3^-/NH_4^+, ln(x+2), n=63

Random effects	Variance
Mesocosm	1.8-05
Residual	0.60

Fixed Effects	Value	Std. error	df	t	p
intercept	1.81	0.21	41	8.58	0.0000
HR-biochar	-0.37	0.30	14	-1.25	0.23
factor (spring)	0.41	0.31	41	1.34	0.19
factor (summer)	-0.36	0.30	41	-1.21	0.23

factor (winter)	-0.05	0.30	41	-0.17	0.86
HR-biochar*factor(spring)	0.03	0.43	41	0.07	0.93
HR-biochar*factor(summer)	0.37	0.42	41	0.89	0.38
HR-biochar*factor(winter)	**1.04**	**0.42**	**41**	**2.47**	**0.02**

NO_2^-+NO_3^-/NH_4^+, ln(x+2), n=63

Random effects	Variance
Mesocosm	1.8-05
Residual	0.62

Fixed Effects	Value	Std. error	df	t	p
intercept	1.90	0.22	41	8.62	0.0000
HR-biochar	-0.35	0.31	14	-1.23	0.28
factor (spring)	0.33	0.32	41	1.02	0.31
factor (summer)	-0.45	0.31	41	-1.44	0.16
factor (winter)	0.09	0.31	41	0.30	0.76
HR-biochar*factor(spring)	-0.001	0.45	41	-0.003	0.99
HR-biochar*factor(summer)	0.35	0.44	41	0.80	0.43
HR-biochar*factor(winter)	**1.02**	**0.44**	**41**	**2.31**	**0.03**

Moisture, ln(x), n=64

Random effects	Variance
Mesocosm	0.02
Residual	0.08

Fixed Effects	Value	Std. error	df	t	p
intercept	2.74	0.03	42	91.49	0.0000
HR-biochar	**0.18**	**0.04**	**14**	**2.29**	**0.0008**
factor (spring)	0.05	0.04	42	1.28	0.21
factor (summer)	-0.38	0.04	42	-9.38	0.0000
factor (winter)	-0.07	0.04	42	4.67	0.0000
HR-biochar*factor(spring)	-0.16	0.06	42	-1.18	0.33
HR-biochar*factor(summer)	**-0.09**	**0.06**	**42**	**-2.74**	**0.009**
HR- biochar*factor(winter)	0.03	0.06	42	0.49	0.63

b. LR- of biochar application

Sodium, ln(x+1), n=64

Random effects	Variance
Mesocosm	0.14
Residual	0.58

Fixed Effects	Value	Std. error	df	t	p

intercept	2.05	0.21	42	9.74	0.0000
LR-biochar	**-0.61**	0.29	14	-2.05	**0.05**
factor (spring)	0.39	0.29	42	1.37	0.18
factor (summer)	0.46	0.29	42	1.59	0.11
factor (winter)	0.001	0.29	42	0.003	0.99
LR-biochar*factor(spring)	**0.89**	**0.41**	**42**	**2.20**	**0.03**
LR-biochar*factor(summer)	0.46	0.41	42	1.14	0.26
LR-biochar*factor(winter)	0.50	0.41	42	1.23	0.22

Calcium, ln(x), n=64

Random effects	Variance
Mesocosm	0.04
Residual	0.46

Fixed Effects	Value	Std. error	df	t	p
intercept	5.09	0.16	42	31.08	0.0000
LR-biochar	-0.25	0.23	14	-1.09	0.29
factor (spring)	-0.13	0.23	42	-0.58	0.56
factor (summer)	-0.39	0.23	42	-1.72	0.09
factor (winter)	0.96	0.23	42	4.17	0.0002
LR-biochar*factor(spring)	**0.63**	**0.32**	**42**	**1.95**	**0.05**
LR-biochar*factor(summer)	0.23	0.32	42	0.70	0.48
LR-biochar*factor(winter)	0.31	0.32	42	0.96	0.34

Chlorine, ln(x), n=64

Random effects	Variance
Mesocosm	0.05
Residual	0.76

Fixed Effects	Value	Std. error	df	t	p
intercept	2.47	0.27	42	9.19	0.0000
LR-biochar	-0.63	0.38	14	-1.65	0.12
factor (spring)	0.03	0.38	42	0.09	0.92
factor (summer)	0.75	0.38	42	-0.35	0.72
factor (winter)	-0.13	0.38	42	6.76	0.0000
LR-biochar*factor(spring)	**0.95**	**0.54**	**42**	**1.77**	**0.08**
LR-biochar*factor(summer)	0.74	0.54	42	1.38	0.17
LR-biochar*factor(winter)	0.89	0.54	42	1.65	0.10

Phosphate, ln(x), n=64

Random effects	Variance
Mesocosm	0.15
Residual	0.43

Fixed Effects	Value	Std. error	df	t	p
intercept	0.83	0.16	42	5.08	0.0000
LR-biochar	0.02	0.23	14	0.07	0.94
factor (spring)	0.84	0.22	42	3.09	0.0003
factor (summer)	1.41	0.22	42	6.51	0.0000
factor (winter)	0.82	0.22	42	3.79	0.0005
LR-biochar*factor(spring)	0.07	0.31	42	0.23	0.82
LR-biochar*factor(summer)	0.31	0.31	42	1.00	0.32
LR-biochar*factor(winter)	**0.63**	**0.31**	**42**	**2.08**	**0.04**

Table S3. Generalized mixed models (GLMM) evaluating the effect of biochar addition on soil biological activity properties (only the models with significant or nearly significant results are shown). a. Linear mixed-effects model fit by REML we carried out in R software (lme4 package) with the formula lme.X<-lme(X~HR-biochar, random=~1|Mesocosm), where HR-biochar corresponds to the biochar application rate comparison between 0 versus 30 t ha^{-1}, and season corresponds to each of the sampled periods during the year (fall 2011, springtime 2012, summer 2012 or winter 2012); and b. correspondingly, in this case linear mixed-effects model with the formula lme.var.<-lme(var.~LD-biochar, random=~1|Mesocosm), where LD-biochar corresponds to the biochar application rate comparison between 0 versus 5 t ha^{-1}. For each model, the transformation method of the response variable is shown together with the number of available observations (n) used for each model construction. Rows in bold highlight statistically significant results ($p<0.05$) regarding biochar amendments.

a. HR- of biochar application

Basal Respiration, untransf., n=63

Random effects	Variance
Mesocosm	7.86e-06
Residual	0.23

Fixed Effects	Value	Std. error	df	t	p
intercept	1.74	0.08	41	21.48	0.0000
HR-biochar	**0.28**	**0.11**	**14**	**2.48**	**0.02**
factor (spring)	-1.02	0.11	41	-8.93	0.0000
factor (summer)	-1.19	0.11	41	-10.40	0.0000
factor (winter)	-1.06	0.11	41	-9.23	0.0000
HR-biochar*factor(spring)	-0.27	0.16	41	-1.62	0.11
HR-biochar*factor(summer)	-0.26	0.16	41	-1.62	0.11
HR-biochar*factor(winter)	0.04	0.16	41	0.25	0.80

16S RNA, $log_{10}(x)$, n=64

Random effects	Variance
Mesocosm	0.04
Residual	0.18

Fixed Effects	Value	Std. error	df	t	p
intercept	9.72	0.06	42	146.24	0.0000
HR-biochar	-0.02	0.09	14	-0.18	0.85
factor (spring)	0.14	0.09	42	1.54	0.13
factor (summer)	0.26	0.09	42	2.80	0.007

factor (winter)	0.25	0.09	42	2.78	0.008
HR-biochar*factor(spring)	0.08	0.13	42	0.64	0.53
HR-biochar*factor(summer)	-0.07	0.13	42	-0.55	0.58
HR-biochar*factor(winter)	**0.22**	**0.13**	**42**	**1.70**	**0.09**

nirS/nirK, untransf., n=64

Random effects	Variance
Mesocosm	4.6e-06
Residual	0.20

Fixed Effects	Value	Std. error	df	t	p
intercept	0.17	0.07	42	2.48	0.02
HR-biochar	**0.18**	**0.10**	**14**	**1.76**	**0.09**
factor (spring)	0.13	0.10	42	1.34	0.18
factor (summer)	0.04	0.10	42	0.44	0.66
factor (winter)	0.07	0.10	42	0.73	0.47
HR-biochar*factor(spring)	-0.21	0.14	42	-1.49	0.14
HR-biochar*factor(summer)	-0.20	0.14	42	-1.43	0.16
HR-biochar*factor(winter)	-0.04	0.14	42	-0.26	0.79

Archeal *amoA* (AOA)/ Bacterial *amoA* (AOB), untransf., n=64

Random effects	Variance
Mesocosm	0.95
Residual	2.60

Fixed Effects	Value	Std. error	df	t	p
intercept	5.31	0.98	42	5.41	0.000
HR-biochar	1.93	1.38	14	1.39	0.18
factor (spring)	-4.03	1.30	42	-3.09	0.003
factor (summer)	-3.94	1.30	42	-3.02	0.004
factor (winter)	-1.79	1.30	42	-1.37	0.17
HR-biochar*factor(spring)	-1.01	1.84	42	-0.55	0.58
HR-biochar*factor(summer)	-1.34	1.84	42	-0.72	0.47
HR-biochar*factor(winter)	**-3.37**	**1.84**	**42**	**-1.83**	**0.07**

b. LR- of biochar application

Metabolic quotient (qCO_2), untransf., n=64

Random effects	Variance
Mesocosm	9.6e-08
Residual	0.001

Fixed Effects	Value	Std. error	df	t	p
intercept	0.004	0.0004	42	10.53	0.0000

LR-biochar	0.0007	0.0006	14	1.16	0.26
factor (spring)	-0.0008	0.0006	42	-1.41	0.16
factor (summer)	-0.0024	0.0006	42	-3.86	0.0004
factor (winter)	0.00002	0.0006	42	0.03	0.97
LR-biochar*factor(spring)	**-0.27**	**0.16**	**42**	**-1.82**	**0.07**
LR-biochar*factor(summer)	-0.26	0.16	42	-1.03	0.31
LR-biochar*factor(winter)	0.04	0.16	42	0.28	0.77

nosZ/nirS+nirK, untransf., n=64

Random effects	Variance
Mesocosm	8.03e-07
Residual	0.04

Fixed Effects	Value	Std. error	df	t	p
intercept	0.09	0.01	42	6.56	0.0000
LR-biochar	0.02	0.02	14	1.36	0.19
factor (spring)	-0.03	0.02	42	-1.79	0.08
factor (summer)	-0.005	0.02	42	-0.29	0.77
factor (winter)	-0.06	0.02	42	-2.95	0.005
LR-biochar*factor(spring)	-0.03	0.03	42	-1.19	0.23
LR-biochar*factor(summer)	**-0.06**	**0.03**	**42**	**-2.24**	**0.03**
LR-biochar*factor(winter)	-0.03	0.03	42	-1.15	0.25

Archeal *amoA* (AOA) / Bacterial *amoA* (AOB), untransf., n=64

Random effects	Variance
Mesocosm	0.81
Residual	4.99

Fixed Effects	Value	Std. error	df	t	p
intercept	5.31	1.78	42	2.97	0.005
LR-biochar	**5.19**	**2.53**	**14**	**2.06**	**0.05**
factor (spring)	-4.03	2.49	42	-1.61	0.003
factor (summer)	-3.94	1.30	42	-1.58	0.004
factor (winter)	-1.79	2.49	42	-0.72	0.17
LR-biochar*factor(spring)	-5.15	3.53	42	-1.46	0.58
LR-biochar*factor(summer)	-5.39	3.53	42	-1.53	0.47
LR-biochar*factor(winter)	**-6.62**	**3.53**	**42**	**-1.88**	**0.06**

nosZ/(AOA+AOB), untransf., n=64

Random effects	Variance
Mesocosm	1.5e-06
Residual	0.13

Fixed Effects	Value	Std. error	df	t	p

intercept	0.43	0.05	42	9.09	0.0000
LR-biochar	**-0.16**	**0.06**	**14**	**-2.47**	**0.02**
factor (spring)	-0.36	0.06	42	-5.48	0.0000
factor (summer)	-0.25	0.06	42	-3.83	0.0004
factor (winter)	-0.27	0.06	42	-4.15	0.0002
LR-biochar*factor(spring)	**0.16**	**0.09**	**42**	**1.71**	**0.09**
LR-biochar*factor(summer)	0.14	0.09	42	1.48	0.14
LR-biochar*factor(winter)	0.15	0.09	42	1.61	0.11

nosZ/Archeal *amoA* (AOA), untransf., n=64

Random effects	Variance
Mesocosm	4.8e-06
Residual	0.17

Fixed Effects	Value	Std. error	df	t	p
intercept	0.53	0.06	42	8.48	0.0000
LR-biochar	**-0.18**	**0.08**	**14**	**-2.13**	**0.05**
factor (spring)	-0.40	0.08	42	-4.65	0.0000
factor (summer)	-0.17	0.08	42	-1.92	0.06
factor (winter)	-0.28	0.08	42	-3.26	0.002
LR-biochar*factor(spring)	0.18	0.12	42	1.46	0.14
LR-biochar*factor(summer)	0.12	0.12	42	0.98	0.33
LR-biochar*factor(winter)	0.15	0.12	42	1.25	0.21

Table S4. Generalized mixed models (GLMM) evaluating the effects of biochar addition on N_2O, CH_4, and CO_2 exchange rates. a. Linear mixed-effects model fit by REML we carried out in R software (lme4 package) with the formula lme.gas<-lme(gas~HR-biochar, random=~1|Event/Mesocosm), where HR-biochar corresponds to the biochar application rate comparison between 0 versus 30 t ha^{-1}, season corresponds to each of the periods during the sampled years (fall 2011, springtime 2012, summer 2012 or winter 2012), and event to each of the 2-4 consecutive days of measurements per season; and b. similarly, in this case linear mixed-effects model with the formula lme.gas<-lme(gas~LD-biochar, random=~1|Event/Mesocosm), where LD-biochar corresponds to the biochar application rate comparison between 0 versus 5 t ha^{-1}. For each model, the transformation method of the response variable is shown together with the number of available observations (n) used for each model construction. Rows in bold highlight statistically significant results ($p<0.05$) regarding biochar amendments.

N_2O (mg m^{-2} h^{-1}),

a. HR- of biochar application, ln(x+10), n=199

Random effects	Variance
Event	9.5e-07
Mesocosm in Event	2.9e-05
Residual	0.39

Fixed Effects	Value	Std. error	df	t	p
Intercept	-0.04	0.08	129	-0.54	0.59
HR-biochar	-0.02	0.11	59	-0.16	0.87
spring	-0.06	0.11	129	-0.58	0.56
summer	0.15	0.11	129	1.43	0.15
fall	-0.04	0.12	129	-0.32	0.75
HR-biochar*spring	0.12	0.15	129	0.83	0.41
HR-biochar*summer	**-0.35**	**0.15**	**129**	**-2.34**	**0.02**
HR-biochar*fall	0.09	0.17	129	0.56	0.58

b. LR- of biochar application, ln(x+10), n=204

Random effects	Variance
Event	0.04
Mesocosm in Event	0.02
Residual	0.06

Fixed Effects	Value	Std. error	df	t	p
Intercept	1.57	0.02	126	74.41	0.00

LR-biochar	0.003	0.02	63	0.17	0.87
spring	-0.002	0.02	126	-0.11	0.91
summer	0.09	0.02	126	4.67	0.00
fall	-0.01	0.02	126	-0.52	0.60
LR-biochar*spring	0.008	0.02	129	0.33	0.74
LR-biochar*summer	-0.004	0.03	126	-0.14	0.88
LR-biochar*fall	0.01	0.03	126	0.45	0.65

CH_4 (mg m^{-2} h^{-1}),

a. HR- of biochar application, ln(x+25), n=197

Random effects	Variance
Event	0.014
Mesocosm in Event	2.2e-05
Residual	0.29

Fixed Effects	Value	Std. error	df	t	p
Intercept	3.24	0.06	127	52.96	0.0000
HR-biochar	-0.02	0.09	59	-0.24	0.81
spring	0.04	0.08	127	0.49	0.63
Summer	-0.06	0.08	127	-0.78	0.43
Fall	-0.02	0.09	127	-0.17	0.86
HR-biochar*spring	0.02	0.11	127	0.19	0.85
HR-biochar*summer	**0.26**	**0.12**	**127**	**2.25**	**0.03**
HR-biochar*fall	0.04	0.13	127	0.30	0.76

b. LR- of biochar application, ln(x+25), n=202

Random effects	Variance
Event	1.88e-07
Mesocosm in Event	1.46e-05
Residual	0.34

Fixed Effects	Value	Std. error	df	t	p
Intercept	3.24	0.06	124	46.86	0.0000
HR-biochar	0.006	0.09	63	0.07	0.95
spring	0.04	0.09	124	0.44	0.66
summer	-0.06	0.09	124	-0.67	0.50
fall	-0.01	0.11	124	-0.13	0.89
HR-biochar*spring	0.01	0.13	124	0.09	0.92
HR-biochar*summer	-0.04	0.13	124	-0.29	0.77
HR-biochar*fall	0.009	0.15	124	0.06	0.95

CO_2 (g m^{-2} h^{-1}),

a. HR- of biochar application, untransf., n=204

Random effects	Variance
Event	4.3e-06
Mesocosm in Event	0.06
Residual	0.16

Fixed Effects	Value	Std. error	df	t	p
Intercept	0.39	0.03	134	11.23	0.0000
HR-biochar	0.03	0.05	59	0.62	0.54
spring	-0.10	0.04	134	-2.33	0.02
summer	0.01	0.04	134	0.33	0.74
fall	0.01	0.05	134	0.29	0.77
HR-biochar*spring	-0.05	0.06	134	-0.85	0.40
HR-biochar*summer	-0.04	0.06	134	-0.74	0.46
HR-biochar*fall	-0.03	0.07	134	-0.40	0.69

b. LR- of biochar application, untransf., n=206

Random effects	Variance
Event	0.014
Mesocosm in Event	0.07
Residual	0.13

Fixed Effects	Value	Std. error	df	t	p
Intercept	0.38	0.03	128	12.73	0.0000
LR-biochar	-0.0004	0.04	63	-0.01	0.99
spring	-0.10	0.04	128	-282	0.005
summer	0.03	0.04	128	0.84	0.40
fall	0.006	0.04	128	0.13	0.89
LR-biochar*spring	-0.04	0.05	128	-0.85	0.40
LR-biochar*summer	-0.07	0.06	128	-1.15	0.26
LR-biochar*fall	-0.09	0.06	128	-1.45	0.15

Figure S1. Mean soil water-soluble content of other ions (see Table S1 for the GLMM probability values), dissolved organic carbon (DOC) and pH during the experiment in the different biochar addition treatments.

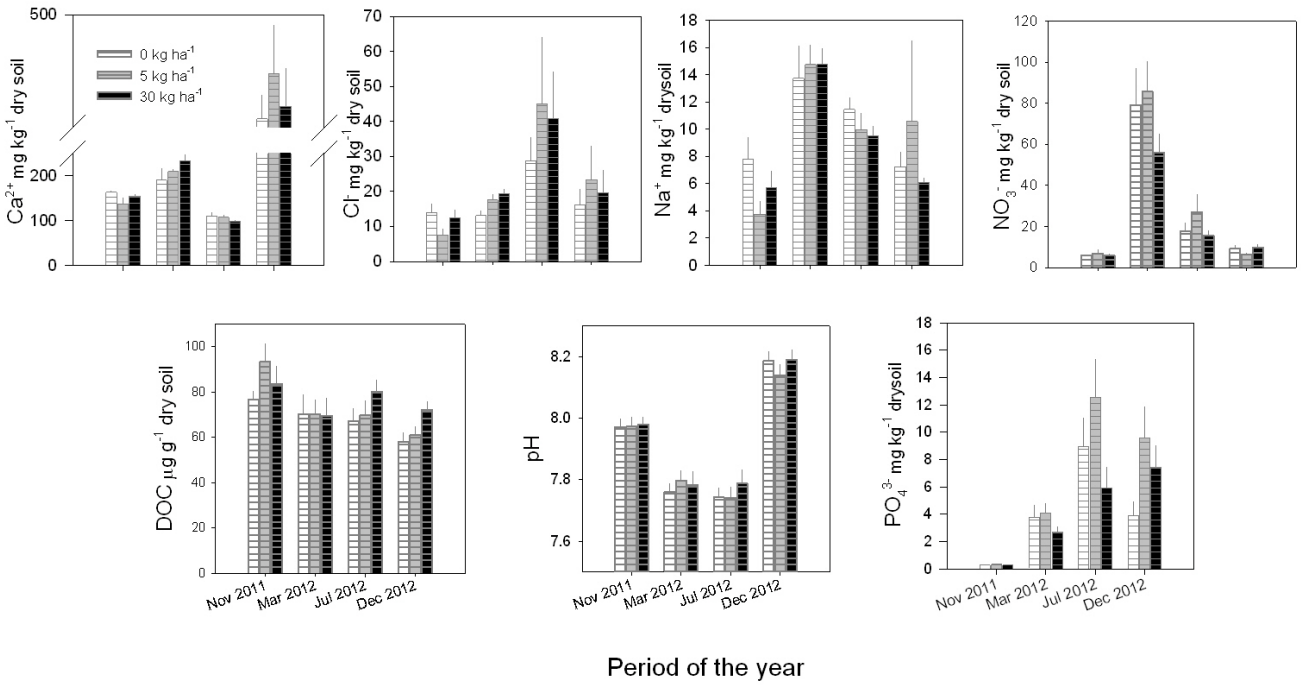


Figure S2. Abundance of bacterial gene copies during the experiment in the different biochar addition treatments.