

Biochar's role in mitigating soil nitrous oxide emissions: A review and

More than two thirds of global nitrous oxide (N₂O) emissions originate from soil, mainly associated with the extensive use of nitrogen (N) fertilizers in agriculture. Although the interaction of black carbon with the N cycle has been long recognized, the impact of biochar on N₂O emissions has only recently been studied. Herein we reflect on proposed hypotheses to explain N₂O decrease with biochar, linking them to specific mechanisms for N₂O formation and consumption in soil. Moreover, to assist in elucidating key mechanisms in which biochar may act in mitigating emissions of N₂O, we undertook a meta-analysis using published literature from 2007 to 2013. This quantitative analysis used 30 studies with 261 experimental treatments. Overall, we found that biochar reduced soil N₂O emissions by 54% in laboratory and field studies. The biochar feedstock, pyrolysis conditions and C/N ratio were shown to be key factors influencing emissions of N₂O while a direct correlation was found between the biochar application rate and N₂O emission reductions. Interactions between soil texture and biochar and the chemical form of N fertilizer applied with biochar were also found to have a major influence on soil N₂O emissions. While there is clear evidence that, in many cases, emissions of N₂O are reduced, there is still a significant lack in understanding of the key mechanisms which result in these changed emissions. As such, we have guided readers with suggestions to address specific research gaps, which we anticipate will enhance our knowledge and understanding of biochar's N₂O emission mitigation potential.

1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas and the single most important ozone depleting compound currently emitted to the atmosphere (Ravishankara et al., 2009). Its atmospheric concentration has increased from 270 parts per billion by volume (ppbv) in the pre-industrial era to ~324 ppbv at present (Ussiri and Lal, 2013). Agriculture is the main source of the global anthropogenic N₂O emissions (Smith et al., 2008), largely due to the widespread use of synthetic nitrogen (N) fertilizers. Although some studies have shown that N₂O can be produced through abiotic redox reactions (Samarkin et al., 2010; Rubasinghege et al., 2011), N₂O is predominantly generated by the microbial transformations of reactive N in soils (Baggs, 2011; Thomson et al., 2012; Butterbach-Bahl et al., 2013). Reactive N (Nr) is defined as organic bound and inorganic N compounds except N₂. Once Nr (organic or mineral) enters the soil, there are numerous processes that can lead to N₂O formation, although their relative importance is still poorly understood (Fernandes et al., 2012; Butterbach-Bahl et al., 2013; Zhu et al., 2013). Three main processes are thought to be the major contributors to N₂O emissions from soils: nitrification, denitrification and dissimilatory nitrate reduction (Baggs, 2011). The relative contribution of each to total N₂O emissions depends not only on the soil characteristics (texture, available carbon, pH, aerobicity, microbial activity) but also on the prevailing environmental conditions (temperature, rainfall). Moreover, soil can act not only as a source but also as a sink for N₂O (Chapuis-Lardy et al., 2007; Thomson et al., 2012). Nitrous oxide can be used by some heterotrophic bacteria, as well as a few autotrophic bacteria for respiration and is further transformed to N₂ (Chapuis-Lardy et al., 2007). The quantity and complexity of N₂O production pathways, and their high spatial and temporal variability, make reducing N₂O emissions from fertilized soils particularly challenging (Venterea et al., 2012). Mitigation of N₂O emissions will ultimately be accomplished by reducing N fertilizer (organic and mineral) inputs, which can only be made viable through increasing the amount of N assimilated by crops via improved N management, where biochar might play a role.

The interaction between biochar and the soil N cycle is acknowledged and there are several comprehensive reviews recently published on this topic (Clough and Condron, 2010; Biederman and

Harpole, 2013; Clough et al., 2013). Applying N fertilizers in combination with biochar has been proposed for improving temporal synchrony between crop-N demand and soil-N availability, enhancing N use efficiency and reducing environmental impacts. However, the mechanisms by which biochar influences such processes are not well understood. The reduction in N₂O emissions after biochar soil amendment was first reported in a greenhouse experiment by Rondon et al. (2005). They found that N₂O emissions were decreased by up to 50% for soybean and by up to 80% for grass growing in a lowfertility oxisol from the Colombian savanna. Since then, the interest in biochar as a N₂O mitigation strategy for agricultural soils has been continuously increasing and the number of studies evaluating N₂O emissions from biochar-treated soils has risen exponentially. Different hypotheses have been postulated to explain why biochar might decrease N₂O emitted from soils. Biochar may enhance soil aeration, increase soil pH, favour N immobilization, interact with available organic carbon and N in soil, modify enzymatic activities as well as potentially induce a toxic effect on nitrifier and denitrifier communities. To date, the hypothetical mechanisms, and the importance they might have in reducing N₂O emissions remain controversial and are not well documented. Similarly, the characteristics that make a biochar most appropriate for N₂O emission mitigation in a particular soil or a range of soils are not well known. Without the basic knowledge of when, how much and why biochar modifies soil N transformation processes and consequently soil N₂O emissions, the use of biochar to mitigate N₂O emissions from fertilized agro-ecosystems remains a difficult challenge. This present review focuses on the impact of biochar on N₂O emissions using meta-analytical techniques to investigate whether the often proposed reductions in N₂O emissions occur across multiple studies. Further, the results of such analysis will be used to provide evidence for proposed mechanisms and highlight the areas where further research is needed.

2. An overview of proposed hypothesis to explain the impact of biochar on N₂O production and consumption pathways in soil

2.1. Abiotic mechanisms

Nitrous oxide can be formed or consumed in soil through abiotically mediated mechanisms (van Cleemput, 1998). The term “chemodenitrification” refers to any abiotic chemical reaction leading to the formation of NO, N₂O and N₂ and includes: (i) chemical decomposition of hydroxylamine (NH₂OH) (Bremner et al., 1980); (ii) chemical decomposition of nitrite (NO₂⁻) (van Cleemput, 1998); and (iii) the recently reported abiotic decomposition of ammonium nitrate (NH₄NO₃) in the presence of light, moisture and reacting surfaces (Rubasinghe et al., 2011). Nitrous oxide can also be abiotically consumed in soil, which implies that, once it is formed (through biotic or abiotic processes) it is further chemically reduced to N₂.

Even though abiotic reactions of N_2O formation and consumption in soil have been rarely investigated, the potential role of biochar as a redox catalyst should not be neglected. In a recent study Cayuela et al. (2013) found no N_2O emissions from a sterilized soil amended with biochar, demonstrating that biochar did not induce abiotic NO_3^- reduction to N_2O through catalytic reactions. However, the abiotic interactions of biochar with other more reactive mineral (NO_2^-) or gaseous N forms (N_2O , NO) in soil still have not been sufficiently explored. Recently, Cornelissen et al. (2013) demonstrated N_2O sorption to anhydrous biochars as a plausible mechanism of N_2O emissions reduction; however the role of water, CO_2 and organic matter in soil as competitors for biochar sorption sites and the fate of biochar-sorbed N_2O in soil, are topics of further research.

Nitrous oxide is a thermodynamically strong oxidant, but kinetically refractory towards decomposition and reduction. This kinetic barrier can be overcome through binding and activation with metal ions such as Fe or Cu (Tolman, 2010). Some recent studies have demonstrated that carbon-based nanomaterials can act as catalytic active sites favouring the transfer of electrons and mediating environmentally relevant redox reactions. For instance Fu and Zhu (2013) showed that graphene oxide acts as a catalyst favouring the reduction of nitrobenzene to aniline and Yuan et al. (2013) found that sewage sludge biochar catalyzes the reduction of oxygen in fuel cell systems. Wannakao et al. (2012) demonstrated that graphene is able to induce the positive charge of an embedded Fe atom, making it ready to react with the N_2O molecule.

As reported by Oh et al. (2013) the microscopic graphene moieties in biochar may serve as both adsorption sites (due to the condensed carbon formed during pyrolysis) and electron-transfer agents. The catalytic ability of biochar can be due to the presence of surface functional groups, such as (hydro)-quinones, or redoxactive metals, such as Fe, Cu and Mn. Cayuela et al. (2013) proposed the function of biochar as an “electron shuttle”, a redox system readily changing from one state to another that favours the transport of electrons to microorganisms. This system has also been described in other forms of organic matter in soil, such as humic substances (Lovley et al., 1996) and microbial exudates (Newman and Kolter, 2000).

Although at present there are no experiments that demonstrate a purely chemical formation or consumption of N_2O in soil after the addition of biochar, many novel studies (such as presented above) provide new and challenging insights into how N_2O might bind to biochars, particularly metal ions embedded in biochar, and be activated for N-N or N-O scission.

2.2. Biotic mechanisms

2.2.1. Biochar liming effect

Soil pH is a master variable affecting N_2O production and consumption processes in soil. Nitrifiers perform well in slightly acidic

to slightly alkaline pH soils (Mørkved et al., 2007), whereas denitrifiers have a wider pH optimum in the range of pH 4–8 (Liu et al., 2010). Therefore, the relationship of nitrification and denitrification on N_2O emissions is not straightforward because of the influence of soil pH on the fractions of transformed N released as N_2O from the two processes (Šimek and Cooper, 2002; Mørkved et al., 2007). Low soil pH may increase the N_2O product ratios for (i) nitrification ($\text{N}_2\text{O}/(\text{NO}_2^- + \text{NO}_3^-)$) possibly due to enhanced denitrification of NO_2^- from nitrification (Mørkved et al., 2007), and (ii) denitrification ($\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$) possibly due to impaired synthesis or activity of the functional N_2O reductase enzyme (Liu et al., 2010; Bakken et al., 2012). These product ratios have been shown to decrease when pH is increased through liming of agricultural soils particularly for denitrification under anaerobic conditions (Mørkved et al., 2007; Liu et al., 2010). However, in drained soils, the pH and N_2O emission relationship may be hampered because liming may transiently increase organic C and N mineralization (Curtin et al., 1998) with potential feedback effects on denitrification and nitrification, respectively (Mørkved et al., 2007). Nevertheless, altering N_2O product ratios of nitrification and denitrification by increasing soil pH within an optimum range is of considerable practical relevance to devise strategies for reducing N_2O emissions from agricultural soils (Šimek and Cooper, 2002).

Biochars are usually alkaline in nature, which may vary in their acid neutralizing (liming) capacity depending on feedstock type and production temperature (Cao et al., 2009; Cao and Harris, 2010; Singh et al., 2010a; Yuan et al., 2011a,b; Yuan and Xu, 2011). Oxygen-containing organic functional groups on biochar surfaces and mineral deposits such as CaCO_3 (Van Zwieten et al., 2010a) formed during pyrolysis or inherited from parent feedstocks are the main forms of alkalinity in biochars, which may neutralize soil acidity (Yuan et al., 2011b). Furthermore, biochars may contain considerable amounts of soluble base cations (Singh et al., 2010a; Yuan et al., 2011b), which can release rapidly into soil. Thus, biochar application may be a novel strategy to increase soil pH, supply base cations, and consequently decrease the N_2O product ratios of nitrification and denitrification (Van Zwieten et al., 2010b; Castaldi et al., 2011). Some studies have linked observed reductions in N_2O emissions to biochar-induced increases in soil pH, which may shift the product stoichiometry of denitrification to increased production of N_2 relative to N_2O (Van Zwieten et al., 2010b). More recently, Cayuela et al. (2013) demonstrated by ^{15}N stable isotope techniques that biochar favours the last step of denitrification, decreasing the $\text{N}_2\text{O}/\text{N}_2$ ratio in 15 soils, and showed that biochar liming effect is a key aspect, but not the only one, for mitigating N_2O emissions during denitrification. In a parallel experiment, addition of CaCO_3 did not decrease N_2O emissions, which demonstrates that a mere shift in soil pH was not the only reason for N_2O mitigation. Further direct field or laboratory-based evidence is required using

¹⁵N stable isotope methodologies to investigate the liming-buffer capacity hypothesis for biochars across predominantly nitrifying and denitrifying conditions.

Biochar may lose its liming effect over time. Furthermore, oxidation of biochar surfaces during ageing may generate acidity and

lower soil pH in the vicinity of biochar particles. Slavich et al.

(2013) reported that the liming effect of animal manure biochar can be retained in soil over several seasons. However, simultaneous field measurements did not show significant reductions in soil

N₂O emissions by the feedlot manure biochar (Scheer et al., 2011).

It is thus important to characterize the longevity of the impact of biochar alkalinity on N₂O product ratios for different biochars and soils, thereby optimizing biochar–soil combinations for reducing soil N₂O emissions.

2.2.2. Impact on soil aeration

The addition of biochar has been reported to decrease soil N₂O emissions by affecting soil physical properties, either by reducing soil compaction and bulk density (Rogovska et al., 2011) or

by adsorbing an excess of soil moisture (Yanai et al., 2007). These

authors reported an increase in soil porosity and aeration which

is a major factor governing N₂O generation and diffusion (Heincke

and Kaupenjohann, 1999). Water-filled pore space (WFPS), a measure of the water–air contents of the soil, regulates soil aeration

and the oxygen availability for microorganisms, affecting the activity of nitrifiers and denitrifiers.

Bateman and Baggs (2005) found

that nitrification was the main process producing N₂O at 35–60%

WFPS, whereas denitrification was the predominant process above

70% WFPS and the major contributor to soil N₂O emissions. Under

severely O₂ limiting conditions denitrifiers can reduce NO₃[–] to N₂

without appreciable N₂O emissions. However, as the availability of

O₂ increases in the soil the amount of emitted N₂O can be enhanced

considerably, increasing the N₂O/N₂ ratio. In this case, nitrifier denitrification may be responsible for the generation of N₂O (Wrage

et al., 2001). With decreasing WFPS, gas diffusion and transport

increases in soil allowing the N₂O to be emitted rather than being

further reduced in the soil (Chapuis-Lardy et al., 2007). Increased O₂ availability at low WFPS inhibits the activity of denitrifiers, with

nitrifiers being the main responsible for N₂O emissions. Despite the

positive impact of biochar in enhancing soil O₂ availability, the net

contribution of biochar in reducing N₂O emissions needs to be carefully assessed since the positive impact of biochar on soil moisture

may lead to an increase of N₂O emissions by stimulating microbial growth (Saarnio et al., 2013). Case et al. (2012) found that the

enhancement of soil aeration by the addition of biochar only made

a minimal contribution to the decrease of N₂O emissions and concluded that biochar should affect

other variables involved in N₂O

production and consumption.

Another indirect impact of biochar on soil porosity and hydraulic

properties is its influence on the formation and stability of soil

aggregates (Liu et al., 2012b; Herath et al., 2013), a subject that has been barely studied to date and that is known to alter greenhouse gas (GHG) emissions (Mukherjee and Lal, 2013).

2.2.3. Interaction with N in soil

Bioavailable or reactive N in soil constitutes both organic and mineral N species, including nitrate and ammonium, which can be readily utilized by microorganisms and plants (Huygens et al., 2008). The availability of this N is the major driver for soil N₂O emissions (Butterbach-Bahl et al., 2013). While there is a recognized lack of literature on the interactions between biochar, soil biota and N cycling (Clough et al., 2013), evidence is emerging that biochar may limit the availability of N in soil, thus reducing substrate availability for production of N₂O.

Biochars produced at high temperatures have been shown to adsorb NO₃⁻ in aqueous sorption experiments (Dempster et al., 2012b; Kameyama et al., 2012; Yao et al., 2012). Clough et al. (2013) reviewed this work in detail, suggesting that biochars produced above 600 °C are more likely to sorb NO₃⁻. Similarly, in soil column experiments, Mukherjee and Zimmerman (2013) presented evidence that higher temperature biochars (650 °C) displayed greater nutrient sorption than low temperature biochars and reasoned that this is due to their greater surface area and organic matter (OM) sorption capacity. However, Kameyama et al. (2012) suggest that this adsorption may be through ionic binding with basic functional groups on the biochar rather than through physical entrapment. Similarly, Knowles et al. (2011) reported significant reductions in NO₃⁻ leaching upon application into soil amended with wood biochar made at 600 °C, but could not determine the mechanisms involved. Case et al. (2012) using wood biochar made at 400 °C and Bruun et al. (2011) using crop residue biochar made at 525 °C also showed a significant decline in soil extractable NO₃⁻ over time in the presence of biochar. Van Zwieten et al. (2010b) reported an accumulation of NO₃⁻ in soils with biochar amendment and a concomitant decrease in N₂O emissions. Here biochar rate tended to influence NO₃⁻ accumulation to a greater degree than biochar temperature or feedstock composition. Similarly, Cayuela et al. (2013) has shown concomitant accumulation of NO₃⁻ in a range of soils with corresponding decrease in emissions of N₂O. Prendergast-Miller et al. (2011) showed accumulation of NO₃⁻ in biochar amended rhizosphere soil, suggesting flow of NO₃⁻ from bulk soil via soil solution, with NO₃⁻ being localized within the pore spaces of biochar in the rhizosphere.

The effects of biochar on NO₃⁻ binding, however, may be short term as biochar surfaces are oxidized in soil, generating acidic (carboxylic and phenolic) functional groups (Cheng et al., 2008; Lin et al., 2012). This is supported by Singh et al. (2010b), who found that biochars produced at 400 and 550 °C did not impact on leaching of NO₃⁻ in two contrasting soil types in a relatively long-term soil incubation study.

While changes to the sorption of mineral N in soil following biochar amendment has been reported in incubation studies, a paucity of data still exists from longer term field studies (Clough et al., 2013). In a 3-year field experiment, contrasting results were observed where a wood biochar appeared to have little effect on the turnover of amino acid-N, the concentration of NH_4^+ or NO_3^- , or the rate of N mineralization in soil (Jones et al., 2012). Evidence suggests that biochars undergo oxidation reactions in soil, which will improve their cation sorption capacity, concomitantly reducing anion exchange. Further, evidence suggests the direct sorption capacity for organic compounds may decrease upon ageing of biochar in soil (Martin et al., 2012), while biochar organo-mineral interactions may increase following ageing (Liang et al., 2010). Biochar application may also positively prime organic matter mineralization in soil (Luo et al., 2011), thus releasing soil organic N from relatively recalcitrant to more labile N forms (Nelissen et al., 2012). Nevertheless, the impact of biochar amendment on the availability of N for microorganisms that produce N_2O remains contradictory. Clearly, further work is required on how biochar properties, as a function of pyrolysis temperature or feedstock type, and its application rate and ageing in soil alter soil NO_3^- and NH_4^+ dynamics and consequently their availability for N_2O emissions.

2.2.4. Interaction with dissolved organic C in soil

Biochar is expected to impact N_2O emission by affecting dissolved organic C availability, which is a major variable governing denitrification potential in soils (Butterbach-Bahl et al., 2013). Denitrification is a facultative process that requires an extra source of organic C as electron donor. Hence the presence of available C would directly promote the growth of denitrifiers but it would also indirectly increase their activity by stimulating soil respiration and reducing O_2 availability in soil (Morley and Baggs, 2010).

The denitrification N_2O product ratio [$\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$] in soils has been reported to be affected by the relative availability of organic C and NO_3^- (Miller et al., 2008) and the quality of the added organic C (Baggs et al., 2000). The application of organic material with a high C/N ratio may lead to decreases in N_2O emissions due to a temporary immobilization of soil N, whereas low C/N ratio materials generally promote rapid mineralization after incorporation in soil leading to higher N_2O emissions (Baggs et al., 2000). Biochar itself does not represent an important source of labile organic C but it is known to interact with native or added organic C, possibly through the sorption of organic compounds on biochar surfaces and incorporation of the added organic C in organo-mineral fractions, reducing the availability of organic C for the soil microorganisms (Joseph et al., 2010; Liang et al., 2010; Keith et al., 2011). Troy et al. (2013) observed that biochar increased N_2O emissions in a soil amended with pig manure, which was attributed to increased WFPS and organic C contents, probably from either the direct input of labile biochar C or through enhanced soil OM mineralization.

The interaction of specific organic C compounds with the denitrifier community, in particular with the regulation of the N₂O reductase enzyme, still remain a research topic requiring further investigation (Morley and Baggs, 2010).

2.2.5. Release of toxic/inhibitory compounds

Several studies have reported an initial noxious effect to plants (Gell et al., 2011) or microbial communities (Dempster et al., 2012a) when certain biochars were applied to soil. Biochar may contain potentially toxic organic compounds (polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo dioxins and furans (PCDDs/Fs)), which develop during the pyrolysis of biomass (Hale et al., 2012; Hilber et al., 2012). The release of these compounds during the first weeks after soil amendment might alter microbial processes. For instance, PAHs have been found to modify nitrification processes (Sverdrup et al., 2002; Maliszewska-Kordybach et al., 2007) and profoundly transform the abundance and structure of denitrifying communities (Guo et al., 2013).

This transient release of inhibitory or toxic compounds after biochar soil amendment has been postulated as a contributing mechanism to explain the reduction of N₂O emissions from soil. Spokas et al. (2010) were the first in reporting a direct impact of a biochar-released microbial inhibitor on GHG decrease. They detected production of ethylene (known to inhibit nitrification and methanotrophic activities) in soils amended with biochar and related this to the observed GHG reductions. More recently, Wang et al. (2013b) reported that the PAHs in low-temperature biochars (300–400 °C) seem to be a dominant factor in reducing N₂O emissions, but not for high-temperature biochars (500–600 °C).

According to Hale et al. (2012) for slow pyrolysis the greatest PAHs concentrations are generally observed in biochars produced between 350 and 550 °C. Biochars produced by fast pyrolysis and gasification contain higher concentrations of PAHs than those produced by slow pyrolysis. In line with this, transient release of inhibitory compounds might be a contributing mechanism for some biochars, however, experiment lags behind theory and there is an urgent need for more studies giving definitive evidence and quantifying the real contribution of this mechanism.

2.2.6. Biochar interactions with the soil biota

While many of the soil biological interactions with biochar remain to be determined, it has been reported that biochar changes microbial abundance and community composition (Lehmann et al., 2011) including ammonium oxidizer community composition (Dempster et al., 2012a). Potentially the ratio of nitrifiers to denitrifiers within the soil will also be affected by biochar application, which has been shown to be the case with biochar applied in compost (Wang et al., 2013a). However, the underlying mechanisms behind such changes in community composition remain largely unknown (Lehmann et al., 2011).

The N-cycle within soils is driven by the microbiota; mainly bacteria and archaea. Some work now exists in the literature regarding the effects of biochar application to soil on the microbiota (Kolton et al., 2011; Chen et al., 2012). However, there is still a paucity of data on the effects of biochar application to soil on microorganisms or genes involved in N-cycling or N₂O production. Ducey et al. (2013) found that biochar increased the abundance of nifH (N fixation), nosZ (nitrous oxide reductase), nirK, and nirS (both nitrite reductase) genes within the microbial communities. All of these genes were found to increase with increasing biochar application rate up to 10% (w:w), the maximum application rate that they studied.

Wang et al. (2013a) reported that N₂O emission rates correlated with the abundance of nosZ, nirK, and nirS genes in compost amended with biochar. They found that the nirK gene (associated with cytochrome cd1 nitrite reductase) was approximately 24 times more abundant than the nirS gene (associated with copper nitrite reductase). Cytochrome cd1 nitrite reductase is also able to reduce molecular oxygen to water. This has the potential to increase O₂ depletion locally and so shift the environment towards one more suitable for denitrification with potential implications for the N₂O/N₂ production ratio. However, the significance of this and whether it occurs in soil following biochar application is yet to be determined.

Biochar amendment to soil has been shown to increase biological nitrogen fixation (BNF) in legumes (Nishio, 1996; Rondon et al.,

2007). This is likely to lead to increase N within the soil system and so increase the potential for N₂O emissions. Several mechanisms have been proposed for the improvement in BNF, including:

increased nodulation (Tagoe et al., 2008; Ogawa and Okimori, 2010; Rillig et al., 2010); immobilization of mineral N (Bruun et al., 2011; Nelissen et al., 2012); and increase P bioavailability (Nelson et al., 2011; Brewer et al., 2012), which can influence N uptake by plants, particularly in P poor soils. However, Saarnio et al. (2013) reported a plant competition effect for N on N₂O fluxes with decreased N₂O emissions when plants were present due to plant uptake competing with microbes for N. The effects of interactions between biochar and BNF on N₂O emissions remain to be determined and are likely to vary between different biochar, plant and soil types.

Little to no work has been conducted on the interaction with biochar and soil mesofauna such as nematodes, collembola and mites. While these organisms play little direct role on the emission of N₂O from soils, microbe feeding mesofauna are known to influence microbial community composition and hence decomposition rates and N mineralization rates (Seastedt, 1984). It is possible that biochar interactions with soil mesofauna can cascade down the soil food web and so affect bacterial and archaeal communities with implications for N₂O emissions from soil. However, further work is needed to test this hypothesis.

Earthworm activity is an important contributor to N₂O emissions from soils (Lubbers et al., 2013). While some work has been published looking at the interactions of earthworms with biochar (e.g. Chan et al., 2008; Weyers and Spokas, 2011; Van Zwieten et al., 2010a), little work has yet been done on the effects on N₂O emissions resulting from the interaction between biochar and earthworms on N cycling. Noguera et al. (2012) found few interactions between earthworms and biochar and no interactions with mineral N. Conversely, Augustenborg et al. (2012) reported that biochar reduced earthworm-induced N₂O fluxes. However, the mechanisms behind this effect were unclear again highlighting the need for further research in this area.

3. Current knowledge: a quantitative analysis of published studies

3.1. Methods

3.1.1. Data sources and compilation

A literature search was conducted using Scopus, Web of Science and Google Scholar databases using the keywords “biochar”

OR “charcoal” OR “black carbon” AND “N₂O” OR “nitrous oxide” OR “greenhouse gas” (cut-off date May 15th 2013). For each article the

title and abstract were evaluated to verify if they reported original quantitative data on N₂O emissions and these articles were examined in detail for quality criteria. A minimum of three replicates per

treatment were required for the study to be included in the metaanalysis. Studies where the gas sampling frequency was considered

not appropriate (gas samples were taken only once or twice during

the entire experiment) were not included. We collected data comparing N₂O emissions between a control and a biochar treatment.

The control was defined as being identical to the treatment for all variables but without biochar.

A total of 261 experimental treatments from 30 peer-reviewed articles published between 2007 and 2013 were selected. Data derived mostly from laboratory or greenhouse experiments (Yanai et al., 2007; Spokas et al., 2009; Spokas and Reikosky, 2009; Cayuela et al., 2010a, 2013; Clough et al., 2010; Singh et al., 2010b; Van Zwieten et al., 2010b; Bruun et al., 2011; Augustenborg et al., 2012; Case et al., 2012; Cheng et al., 2012; Jia et al., 2012; Kammann et al., 2012; Sarkhot et al., 2012; Schouten et al., 2012; Stewart et al., 2012; Zheng et al., 2012; Ameloot et al., 2013; Angst et al., 2013; Malghani et al., 2013; Saarnio et al., 2013; Suddick and Six, 2013; Troy et al., 2013; Wang et al., 2013b), but also from field studies (Scheer et al., 2011; Taghizadeh-Toosi et al., 2011; Liu et al., 2012a; Zhang et al., 2012a,b). Only peer-reviewed studies were included because we did not find studies in the grey literature satisfying our quality criteria. To test the possibility of publication bias (because of studies showing no significant effects being not considered for publication) the Rosenthal's Fail-safe N test was used (Rosenthal, 1979).

From each study relevant data were extracted for (i) soil type (texture, pH), (ii) biochar (feedstock, production temperature and type of pyrolysis (slow, fast, hydrochar), application rate, pH and C/N) and (iii) environmental conditions (field/lab study, soil moisture, rate and type of extra N-fertilizer and study length). In

addition, data on biochar ash concentration and biochar ageing (in soil) were extracted when available. When data were only provided in graphs, Plot Digitizer 2.6.2 was used to extract data points. When no measure of variance was given, or if some relevant data were not included in the paper, efforts were made to contact the corresponding authors to obtain these data.

If such information was not

provided, these studies were excluded from the meta-analysis. We focused our study on soils emitting N₂O. Cases where soil acted as N₂O sink were only found in a forest nursery soil (Spokas and Reikosky, 2009) and were not included in the study. If data from the same experiment, from the same study period, were reported in several papers (e.g. in chronosequence studies with different papers showing data from the same study) the data were only included once from the paper showing the longest study.

3.1.2. Data treatment and definition of categories

Data were subjected to a standardization process to allow for comparisons. Biochar application rates were transformed to percentages of dry weight ratio (w:w) considering the soil layer

reported in each study (or a layer of 20 cm when not reported) and the bulk density (BD) of the soil. If BD was not included in the article, it was calculated from the soil texture according to (Saxton et al., 1986). The pH values measured with CaCl₂ were transformed with the formula $\text{pH}[\text{H}_2\text{O}] = 1.65 + 0.86 \times \text{pH}[\text{CaCl}_2]$ in order to make them comparable to pH measured in distilled water (Biederman and Harpole, 2013). When the temperature of pyrolysis was given as a range (e.g. 400–500 °C), the average value was chosen (i.e. 450 °C). Soil moisture was found and reported as both percentage of water holding capacity (WHC) or water filled pore space (WFPS). The database containing the data for the meta-analysis is included as supplementary material.

The selected data were grouped by categories. Feedstocks were grouped in five categories: (i) biowaste (municipal solid waste), (ii) biosolids (sewage sludge from water treatment plants), (iii) manures or manure-based materials (from poultry, pig or cattle), (iv) wood (oak, pine, willow, sycamore and unidentified wood mixtures), (v) herbaceous (greenwaste, bamboo, straws) and (vi) lignocellulosic waste (including rice husk, nuts shells, papermill waste). The selection was aimed at producing homogenous groupings representative of the main feedstock types utilized for biochar

production. Biochars were grouped according to their pyrolysis production temperature (≤ 400 , 401–500, 501–600, >600 °C) and the pyrolysis type (slow, fast or hydrochar). pH ranged from 4.2 to 9.0 in soils, being mostly acidic and neutral, and from 4.2 to

10.5 in biochars, being predominantly alkaline. Due to the different data distributions they were grouped differently: two levels of acidity for soils (<5 and 5–6.5) and two levels of alkalinity for biochars (7.5–9.5 and >9.5). Soil texture was grouped into four categories: (i) organic soil (organic C >12%, w:w, basis), (ii) coarse (sandy loam, sandy clay loam, loamy sand), (iii) fine (clay, silt clay, sandy clay) or (iv) medium (clay loam, loam, silty clay loam, silt, silt loam) (USDA, 1999). Soil moisture was classified as: (i) “low”, when WFPS or WHC were lower than 80%, (ii) “high”, when WFPS or WHC equal or higher than 80% or as (iii) “cycles”, when soil moisture was subjected to drying-rewetting cycles in microcosms, pots or field studies. In some studies biochar was added in combination with N fertilisers. These were grouped as: urea, nitrate (NO_3^-), organic amendment (compost, manure), ammonium nitrate (NH_4NO_3). Finally biochar application rate was subdivided in five categories (<1, 1–<2, 2–<5, 5–<10, $\geq 10\%$, dry weight ratio (w:w) basis).

3.1.3. Meta-analysis

Standardization of the literature results on the impact of biochar application on N_2O emissions was quantified through a metaanalysis (Rosenberg et al., 2000). For each study the control and experimental (biochar) means were recorded (as average N_2O fluxes or total cumulative N_2O emitted). The standard deviations of both control and biochar treatments were included as a measure of variance. We used the natural log-transformed response ratio (RR) as a measure of effect size for the meta-analysis: $\ln \text{RR} = \ln \frac{\text{XB}}{\text{XC}}$ where XB represents the mean of the biochar treatment and XC the mean of the control group. The effect sizes of each grouping were calculated using a categorical random effects model, where the effect size is weighted in inverse proportion to its variance (Adams et al., 1997). Groups with less than two treatments were excluded from the analysis. Mean effect sizes of each category and the 95% confidence intervals generated by bootstrapping (999 iterations) were calculated with MetaWin Version 2 Statistical software (Rosenberg et al., 2000). In the graphs, the change in N_2O emissions is shown as a percentage of the control (the effect size was exponentially transformed and multiplied by 100 to obtain the percentage change).

3.2. How do biochar characteristics influence N_2O mitigation?

Biochar is not a single “uniquely defined” substance but includes a series of charred biomass materials with different chemical composition and varying degrees of aromaticity (Keiluweit et al., 2010).

Biochar physical architecture and molecular composition depend on both the original feedstock and the pyrolysis conditions. For instance, Zhao et al. (2013) found that, for the same pyrolysis conditions, properties including: fixed carbon, surface area, mineral constituents or chemical functional groups widely differed depending on the feedstock. Keiluweit et al. (2010) proposed a gradual

change in the molecular structure of biochars with increasing temperatures and defined four categories of biochars. The transition temperature of one category to another depends on the pyrolysis conditions (rate of temperature increase, time at highest treatment temperature (HTT) and exposure to O₂ /cooling rate) and initial feedstock (biomass components and physical structure). This gives an idea about the complexity of the subject but, at the same time, it provides possibilities for developing biochars for specific scenarios with the ultimate aim of reducing soil N₂ O emissions.

3.2.1. Feedstock

Fig. 1 shows the mean effect size in N₂ O emissions grouped by the original feedstock class. The sample mean shows a general strong and significant reduction ($-54 \pm 6\%$) in N₂ O emissions. The largest number of studies has been undertaken on wood (415 direct comparisons) and herbaceous materials (282 direct comparisons) and in both cases the mean change (reduction) in N₂ O emissions was significant. There were a considerable number of studies on manure-based biochars (107 direct comparisons), with the mean effect size close to zero, with a confidence interval from -46% to $+39\%$. The number of studies on biowaste and biosolids was considerably lower, and limited to only one or two temperatures of pyrolysis and soil type. Hence, the results were highly variable and more studies are needed to define their influence. From the studies carried out to date, the combination of chemical composition (e.g. N content, aromaticity) and physical properties (e.g. surface area, porosity) related to the specific feedstock possibly defined the potential of a certain biochar to decrease soil N₂ O emissions. However, we could only test a limited number of biochar chemical properties (Section 3.2.3), since a complete physico-chemical pyrolysis process, the degree of carbonization is a continuum of temperature, residence time, atmosphere and particle size of the feedstock being pyrolysed (Joseph et al., 2010). The majority of studies used for our meta-analysis did not accurately report the conditions for pyrolysis, so these parameters could not be tested. Both fast and slow pyrolysis biochars exhibited significant reductions in N₂ O emissions, although fast pyrolysis exhibited a higher variability (Fig. 2b). The number of studies with hydrochar was very low, being the only biochar type where no significant effects on N₂ O emissions were observed.

characterization was missing in most of the studies used for the meta-analysis. Plant derived materials seem to be the most promising feedstock in mitigating N₂ O emissions but more studies are needed for other important group of feedstock for which scarce or no information is currently available.

3.2.2. Temperature and type of pyrolysis

No significant differences were found among the different temperatures of pyrolysis in terms of the extent of N₂ O emissions mitigation although the variability was higher for the $<400^\circ\text{C}$ and

>600 °C biochars (Fig. 2a). It is important to note that although temperature is normally used as an important parameter defining the

3.2.3. Biochar chemical properties (C/N, pH, ash)

Low and very low C/N biochars (C/N < 30), from a variety of original feedstocks (herbaceous, manures, peanut hulls) did not significantly affect N₂O emissions, while for C/N ratios higher than 30, there was a significant reduction in N₂O emissions (Fig. 3). The means for the different C/N ratio classes with values higher than 30 were close to the grand mean and there were no significant differences between them.

Application of high C/N residues to soil has been found to decrease N₂O emissions due to microbial N immobilization (Baggs et al., 2000; Cayuela et al., 2010b). However, this mechanism may be less important for biochar because of its predominantly aromatic structure, thus decreasing bioavailable C for inducing N immobilization (Major et al., 2010; Singh et al., 2012). The lower mitigation

capacity of high-N biochars (e.g. manures or biosolids) is probably due to the increased N release in soil from the biochar (Singh et al., 2010b; Schouten et al., 2012; Wang et al., 2012). Even if N in biochar is occluded in aromatic structures, a small percentage (10–20%) of N has been found to be mineralized (Schouten et al., 2012).

Grouping by biochar pH or ash concentration did not lead to differences in N₂O mitigation (Supplementary Figs. 1 and 2).

Although pH is known to affect nitrification and denitrification rates and associated N₂O product ratios, biochar pH is not the most suitable parameter defining its acid buffer capacity. As previously mentioned, different oxygen-containing organic anions on biochar surfaces and mineral salts such as CaCO₃ provide alkalinity to biochars. The buffer capacity of a certain biochar will

depend on the type and concentration of alkalis present (Yuan et al., 2011b). In addition, depending on the soil, biochar application might not significantly influence soil pH. Most studies included in the meta-analysis did not report the changes in soil pH after biochar application, so we were not able to include this factor in the metaanalysis. Clearly, further research is warranted to elucidate the role of soil pH changes through biochar on N₂O emission mitigation.

Ash concentration was included because high-salt biochars could induce a “salting out effect” leading to higher N₂O emissions (Heincke and Kaupenjohann, 1999), which might reduce the capacity of these biochars for mitigating N₂O emissions. Cayuela et al. (2013) found a negative correlation between N₂O mitigation and ash concentration in a study with nine biochars under denitrification conditions. However, other processes could have simultaneously operated and the importance of biochars salt concentration needs further research.

3.2.4. Biochar application rate

There was a clear correlation between the biochar application rate and the observed decrease in N₂O emissions (Fig. 4). An application rate of between 1 and 2% (dry weight basis) was sufficient

to significantly reduce the N₂O emitted (mean reduction of 27%) whereas high doses (>10%) reduced emissions up to 87%. This shows that a decrease in N₂O emissions by biochar can be a function of biochar properties and their impacts on soil properties, which are additive and may become pronounced with their increasing content and dominance in soil.

3.3. How do soil characteristics and N fertilizer type influence biochar N₂O mitigation capacity?

Some authors have reported contrasting impacts on N₂O emissions when the same biochar was applied to different soils (Spokas and Reikosky, 2009; Stewart et al., 2012; Yoo and Kang, 2012; Zheng et al., 2012). This is clearly linked to different N₂O production pathways operating in contrasting soils, where biochar would differently influence these processes.

3.3.1. Soil texture

The mean change in N₂O emissions in response to different soil textures was analysed separately for high moisture (>80% of the WFPS) and low moisture (<80% of the WFPS) conditions (Fig. 5a and b). Under high moisture, biochar had the greatest mitigation of N₂O emissions in fine texture soils, which are more prone to denitrification. However, for coarse soils biochar did not exhibit a significant effect, despite the mean showing a 53% increase in N₂O emissions after biochar application, due to the high level of variance in the data. For low moisture conditions the trend was opposite and coarse texture soils led to the greatest mitigation of N₂O emissions. There were not enough field (or other drying-rewetting) studies to allow for analysis of how soil texture influences biochar effectiveness to reduce emissions under those conditions.

3.3.2. Soil pH

Biochar was not effective at mitigating N₂O emissions in acid soils (pH < 5), where there was no significant difference with the control (Fig. 6). The impact of biochar was maximal in soils close to neutrality but it did not differ significantly with slightly acidic or alkaline soils. Soil pHs lower than 5 can adversely affect the activity of nitrous oxide reductase (Liu et al., 2010) and it is possible that biochar application could not consistently alleviate the adverse effect of such acid pHs.

3.3.3. Chemical form of applied N-fertilizer

Another aspect that might influence the effectiveness of biochar for reducing N₂O emissions is the chemical form of N (i.e. fertilizer) co-applied with biochar (Fig. 7). Addition of biochar in combination with organic amendments or NH₄NO₃ led to non-significant changes in N₂O emissions with respect to the control. The mechanisms behind these interactions require further research. The significantly lower N₂O mitigation with NH₄NO₃ compared to only NO₃ – seems paradoxical. However, this might be related to the abiotic photo-reduction of nitrate coupled to the oxidation of NH₄⁺, mechanism that has been recently reported by Rubasinghege et al. (2011), where biochar might act as a reactive surface activating the

process.

The greatest effect mean was observed when biochar was applied with NO_3^- (80% decrease), which seems to point to an effect on the denitrification process.

4. Knowledge gaps and future challenges

To date, most studies published on biochar and N_2O emissions are exploratory, reporting differences between a control and a biochar-treatment and providing insights into the impact of biochar on soil properties and conditions relevant for N_2O emissions, but without paying much attention to the different mechanisms involved. Many quantitative data have been published already, and our analysis shows that differences in N_2O emissions between the control and biochar treatments do exist, but that they are highly contextual.

The next generation of studies on biochar and N_2O emissions needs to focus on a mechanistic understanding of the interactions of biochar in soil. Some basic information that should be systematically included are (i) the characteristics of original feedstock (origin and chemical characterization); (ii) the pyrolysis conditions (the type of reactor, temperature ramp, highest treatment temperature, exposure time, atmosphere, level and type of activation) (iii) the physico-chemical characterization of the biochar (particle size, proximate analysis, pH, acid neutralizing capacity: CaCO_3 equivalents, electrical conductivity, bulk density, surface area, adsorption capacity, elemental analysis (C, N, H, O, S), potential toxicity) and (iv) the soil characteristics (texture, bulk density, pH, total and soluble organic C, total N, mineral N). Specific studies should also look at microbial C and N, soil functionality tested by enzyme activities, and microbial community composition.

There is an urgent need to identify the mechanisms underpinning N_2O emission mitigation with biochar and to quantify the contribution of each mechanism to total N_2O reductions. With this purpose, the design of specific experiments focusing on key processes (and preventing possible confounding effects) is essential.

The use of novel tools including isotopes, inhibitors and molecular techniques, recently reviewed by Butterbach-Bahl et al. (2013), should be gradually implemented to characterize and quantify soil processes in biochar- N_2O studies.

Although here we were able to identify some factors (e.g. feedstock type, production temperature and process, biochar and soil properties, application rate, biochar N-source interactions) contributing to reductions in N_2O emissions with biochar (and associated variability in the observed effects), several other potentially important factors could not be tested owing to a lack of appropriate studies. For instance, we were not able to evaluate the impact of biochar on N_2O emissions which are dependent on soil total organic C as this parameter was often not presented in published manuscripts. Another important factor to consider (that has been insufficiently addressed) is the interaction of biochar with

plants. An increase in plant productivity has been observed in soils amended with biochar (Jeffery et al., 2011). Similarly, in some studies biochar has been found to favour BNF, reduce N uptake by plant or minimize NO_3^- leaching and therefore increase N concentration in soil. In this sense, N_2O emissions should be assessed as a function of crop N uptake and crop productivity (expressed as above-ground N uptake: 'yield-scaled N_2O emissions' (Van Groenigen et al., 2010)) instead of merely reporting total emissions.

Finally, another important uncertainty is how ageing will affect the ability of biochar to decrease N_2O emissions. A reduction in the impact of biochar on soil N_2O emissions has been observed after three years of ageing in soil (Spokas, 2012). However, the cause of the loss of N_2O mitigation ability of biochar is unknown, as is the impact of these phenomena across a range of soil types. Determining the mechanism responsible for this loss and the actual duration of the mitigation effect is fundamental to establish the best management practices incorporating biochar.