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Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis

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ABSTRACT

The interaction between biochar and soil changes nitrogen (N) dynamics in different ecosystems. Although multiple studies have reported influences of biochar on soil inorganic N (SIN) including ammonium (NH_4^+-N) and nitrate (NO_3^--N), the influences reported are contradictory. We undertook a meta-analysis to investigate how biochar properties and the interaction among biochar, soil and fertilisation affect SIN. This quantitative analysis used 56 studies with 1080 experimental cases from manuscripts published between 2010 and 2015. Overall, we found that biochar reduced SIN regardless of experimental conditions (approximately $-11 \pm 2\%$ of NH_4^+-N and $-10 \pm 1.6\%$ of NO_3^--N); however, 95% of cases were observed within one year after biochar application. SIN was best explained by residence time of biochar in soil, pyrolysis temperature, application rate, fertiliser type, and soil pH. The effects of biochar were complex due to the interaction of biochar with environmental factors. Most biochar trials used wood as a feedstock, but woody biochar did not decrease SIN as much as other plant-derived biochars. When biochar was used with NH_4-based fertilisers, SIN decreased compared to biochar with no fertiliser. In contrast, adding organic fertiliser with biochar increased SIN compared to biochar alone. SIN was clearly reduced after one month of biochar application, suggesting that biochar should be applied at least one month prior to planting so plants are not affected by decreased N. Our results revealed that the interactions between biochar and environmental factors, pyrolysis temperature of biochar and biochar surface properties are the main driving factors affecting SIN. There were limited long-term studies of >1 year, thus the long-term effects of biochar on SIN still remain unclear.

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1. Introduction

Nitrogen (N) is one of the most critical elements for plant growth and productivity (Atkinson et al., 2010; Bai et al., 2012; Reverchon et al., 2014; Bai et al., 2016). In particular, soil inorganic nitrogen (SIN) is an important N source for plants because plants uptake inorganic N directly through the rooting system (Lynch, 1995). However, N loss via leaching and volatilisation leads to reduced crop productivity, eutrophication, excess nitrate in groundwater, and increased nitrous oxide (N_2O) emissions (Overrein, 1969; Bradbury et al., 1993; Xing and Zhu, 2000; Mikkelsen and Hartz, 2008).

Biochar is a promising soil additive to reduce N loss and improve soil fertility (Lal, 2009; Joseph et al., 2013). Biochar is a carbon (C) rich material produced by pyrolysis of biomass at relatively low temperatures (<700 °C) without oxygen (O_2) (Lehmann and Joseph, 2009). There are contradictory reports regarding N availability when biochar is applied including decrease, increase and no effect (Blackwell et al., 2009; Clough et al., 2013; Bai et al., 2015b; Xu et al., 2015). However, these studies have not yet been synthesised; therefore, a systematic analysis of the relationship between biochar and SIN is necessary. This study aimed to (a) investigate possible mechanisms influencing SIN when biochar is applied through a short review of available published studies and (b) use a meta-analysis to explore the general trends in NH_4^+-N and NO_3^--N across multiple studies in the presence of biochar.

2. Overview of the mechanisms affecting soil inorganic N after biochar application

2.1. Abiotic mechanisms - adsorption/desorption

Chemisorption of SIN by biochar is based on functional groups (Lehmann and Joseph, 2009). Acid functional groups include carboxylic, hydroxyl, lactone and lactol groups on the surface of biochar (Brennan et al., 2001; Amonette and Joseph, 2009). Carboxylic groups are strong Bronsted acids; less acidic groups include phenols and carbonyls. They have a negative charge and adsorb NH_4^+-N by electrostatic attraction (Montes-Morán et al., 2004; Zheng et al., 2010). In general, NO_3^--N adsorption by biochar is weak because biochar carries greater negative surface charges than positive surface charges (Kameyama et al., 2012). However, the existence of base functional groups including chromenes, ketones and pyrones on biochar can facilitate NO_3^--N adsorption to biochar (Montes-Morán et al., 2004; Amonette and Joseph, 2009). NO_3^--N adsorption is also possible via unconventional H-bonding between NO_3^--N ions and the biochar surface (Mukherjee et al., 2011; Lawrinenko, 2014; Kammann et al., 2015).

SIN adsorption by biochar is also time dependent and varies with the temperature and feedstock used to produce biochar. Over time, oxygen-containing acid functional groups (e.g. carboxyl and hydroxyl) are formed on the biochar surface leading to increased biochar CEC and the potential to adsorb more NH_4^+-N than fresh biochar. During the ageing of biochar in the soil, NO_3^--N chemisorption through H-bonding may be enhanced because biochar becomes more hydrophilic (Hammes and Schmidt, 2009). The effect of feedstock on NO_3^--N adsorption and the mechanisms of NO_3^--N adsorption by aged biochar through H-bonding remain unclear and need further research (Clough et al., 2013; Kammann et al., 2015).

Sorption capacity of biochar decreases with increased pyrolysis temperature (Mukherjee et al., 2011; Gai et al., 2014). At high temperatures (>600 °C), acidic functional groups (mainly carboxyl) are converted to neutral or basic fused aromatic groups due to the loss of oxygen-containing functional groups, leading to decreased CEC (Cheng et al., 2008; Gaskin et al., 2008; Kookana et al., 2011; Gai et al., 2014). Therefore, aged biochar produced at lower temperatures are expected to adsorb more NH_4^+-N compared to the fresh and high temperature biochar.

The feedstock used to produce biochar influences acid (carboxylic) functional groups in biochar to adsorb NH_4^+-N (Kookana et al., 2011). For example, grassy biochars (produced from cordgrass) have a higher concentration of carboxylic groups than woody biochars (produced from honey mesquite and loblolly pine), and thus the sorption capacity of the grassy biochars is higher than the woody biochar (Harvey et al., 2012). The higher concentration of carboxylic functional groups is probably because of a high concentration of cellulose, alkali salts and alkali metal oxides in their feedstock (Harvey et al., 2012). Lignocellulose fragments in grassy feedstocks are oxidised more efficiently during pyrolysis and cycloreversion oxidation occurs more rapidly to carboxylic acids. These processes are less efficient in woody feedstocks, owing to a reduced surface charge at any pyrolysis temperature compared with grassy feedstocks (Harvey et al., 2012).

Additionally, physisorption happens inside the pores and on the inner surface of biochar (Lehmann and Joseph, 2009; Saleh et al., 2012; Clough et al., 2013). The inner-surface area of biochar has a positive correlation with the adsorption capability of biochar (Zhang et al., 2012). A commonly used surface area measurement is BET surface area, calculated by determining the adsorption of gases in multi-molecular layers (Brunauer et al., 1938). For example, the BET surface area of biochar from beet-root chips and spent brewer's grains is 10 times higher than BET of hydrochar from the same feedstocks, leading to increased NH_4^+-N adsorption due to increased physisorption (Bargmann et al., 2014). Generally, biochars produced from high temperatures and slow pyrolysis possess higher BET and pore volume, and have a higher physisorption capacity (Lua et al., 2004; Downie et al., 2009; Kookana et al., 2011; Bruun et al., 2012). At high temperature (>600 °C), biochar surface area and pores are enhanced due to the enhancement of crystallites and their ordered structure (Downie et al., 2009). However, when the temperature reaches a threshold (e.g. pine biochar at 750 °C, wheat residue biochar at 700 °C), deformation occurs, micropore structure is destroyed and surface area decreases (Chun et al., 2004; Brown et al., 2006; Downie et al., 2009). Slow pyrolysis conditions also enhance BET, for example BET of wheat straw biochars are 0.6 and 1.6 $m^2 g^{-1}$ for fast and slow pyrolysis, respectively (Bruun et al., 2012). The longer retention times of slow pyrolysis enables micropores to form (Downie et al., 2009).

Some studies report that some of the adsorbed N compounds could be desorbed over time and become available (Rosa and Knicker, 2011; Kameyama et al., 2012; Taghizadeh-Toosi et al., 2012a). Desorption of SIN probably depends on SIN adsorption capacity of biochar which is related to biochar properties, biochar application rate, evolution of soil anion and cation exchange capacity, N loading capacity of ecosystems, soil hydraulic characteristics, climate conditions, soil type, and N demands of plants and microorganisms (Clough et al., 2013).

2.2. Biotic mechanisms

2.2.1. Mineralisation

Biochar can increase, decrease or have no effect on the conversion of organic N to inorganic N (soil N mineralisation) (Hart et al., 1994; Kuzyakov et al., 2009; Spokas et al., 2010; Zimmerman et al., 2011; Singh and Cowie, 2014). Biochar effects on N mineralisation depend on feedstock, pyrolysis temperature, time after application, and biochar C:N ratio (Chan and Xu, 2009; Zavalloni et al., 2011; Zimmerman et al., 2011; Clough et al., 2013).

Priming effect, the change in the mineralization of native soil organic matters (SOM) due to the addition of new substrates, could be affected by biochar amendment (Zimmerman et al., 2011). Different pyrolysis temperatures and feedstock can modify the priming effect of biochar because temperature and feedstock influence the content of biochar labile organic compounds, which determine the rate of the priming effect (Luo et al., 2011; Zimmerman et al., 2011; Singh et al., 2012). For example, biochars produced at low temperatures (<400 °C) lead to a higher mineralisation rate than those from high temperature (>525 °C) due to higher readily decomposable organic C content (Luo et al., 2011; Zimmerman et al., 2011; Singh et al., 2012). Manure biochar is likely to stimulate N mineralisation more than plant-based biochars owing to the lower C:N ratio (Singh et al., 2012). Generally, a high N feedstock stimulates N mineralisation (Thies et al., 2015).

Mineralisation is a time-dependent process (Zimmerman et al., 2011). The priming effect caused by biochar is observed immediately after application (Luo et al., 2011), or shortly after application (90 days) (Zimmerman et al., 2011). The priming effect on N mineralisation is generally a short-term event (Zimmerman et al., 2011; Naisse et al., 2015). The positive priming effect happens initially because biochar as a new C source stimulates soil microbes to mineralise biochar-labile organic compounds; consequently, the remineralisation and co-metabolisation of SOM occur (Kuzyakov et al., 2000; Kuzyakov et al., 2009; Singh and Cowie, 2014). However, in the long term (e.g. 250–500 days in Zimmerman et al., 2011), N mineralisation decreases because organic matters are adsorbed on biochar surfaces, or in soil pores to make them less accessible for microorganisms. As a result, they become unavailable to soil microorganisms as a nutrient source and the mineralisation rate decreases.

Briefly, N mineralisation is a short-term process; therefore potential increases of SIN due to the biochar priming effect may not be detected if soil is not examined soon after biochar application. It is also unclear to what extent different biochars can increase N mineralisation when they interact with soils and fertilisers. Further long-term studies of biochar and native SOM are necessary to understand the effects of biochar on N mineralisation.

2.2.2. Immobilisation

Both NH_4^+-N and NO_3^--N can be assimilated by microbes and be unavailable for plant uptake through microbial immobilisation, the uptake of inorganic N by microorganisms (Hart et al., 1994). Soil microorganisms use labile organic compounds in fresh low-temperature biochar as a nutrient source for immobilisation (Smith et al., 2010; Bruun et al., 2011; Clough et al., 2013). Compounds containing acid-hydrolysable N (e.g. amino sugars, amino acids) in biochars are used readily by soil microorganisms (Rosa and Knicker, 2011; Wang et al., 2012). Such immobilisation can occur in as little as 10 days after biochar application (Bruun et al., 2011; Zhang et al., 2012). The evidence of enhanced N immobilisation is the combination of SIN decline and the increase in microbial abundances associated with N cycling and CO_2 emissions due to microbial respiration (Deenik et al., 2010; Lentz and Ippolito, 2011; Zhang et al., 2012; Ducey et al., 2013). There are several factors affecting N immobilisation including feedstock, C:N ratio of biochar, C:N ratio of co-amended organic substrate (see Section 2.2.1 of this article), and the abundance of substrates that can be used by soil microorganisms (Zavalloni et al., 2011). Woody biochar is assumed to stimulate N immobilisation but the extent of stimulation remains uncertain because woody biochar is more recalcitrant than other biochars (Lehmann et al., 2006; DeLuca et al., 2015).

The C:N ratio of biochar determines whether biochar will trigger N immobilisation or mineralisation (Chan and Xu, 2009). The C:N of 20 is the threshold; if the ratio is above 20, N immobilisation occurs; when the ratio is lower than 20, N mineralisation happens (Chan and Xu, 2009). In reality, this threshold ranges between 20 and 32 (Kuzyakov et al., 2000; Sullivan and Miller, 2001). The C:N ratio of the soil also can be used to explain the priming effect; soil C:N over 32 stimulates N immobilisation (Novak et al., 2010; Zavalloni et al., 2011; Bruun et al., 2012; Xu et al., 2015).

2.2.3. Nitrification

The addition of biochar into the soil may moderate soil temperature, enhance soil moisture and aeration, and thus stimulate nitrifier activities (Mukherjee and Lal, 2013; Zhang et al., 2013; Ulyett et al., 2014). Biochar reduces diurnal and seasonal temperature variations due to the combined modification of soil thermal conductivity and reflectance (Zhang et al., 2013). Biochar retains water in its pores, reduces soil bulk density and increases soil porosity, adsorbs nitrifier inhibitors from soil (e.g. phenolics), and thus stimulates nitrification (Berglund et al., 2004; Gundale and DeLuca, 2006; Joseph et al., 2009; Mukherjee and Lal, 2013). In general, biochar application may activate nitrifiers including ammonia oxidising archaea (AOA) and ammonia oxidising bacteria in aerobic conditions (Thies et al., 2015). However, in water-saturated conditions, there is no correlation between AOA, AOB and biochar application (Harter et al., 2014; Thies et al., 2015). In aerobic field soil, gene copy numbers of AOA increased by 1.5 times and AOB increased by 1.7 times when amended with biochar and NPK fertiliser, corresponding with the increased net nitrification rate in amended soil (Prommer et al., 2014; Bai et al., 2015a). However, it may take over one year for biochar to provide habitat for nitrifiers to colonise (Prommer et al., 2014; Bai et al., 2015a). Similar results have been obtained in aerobic laboratory experiments (Song et al., 2014). Redox reactions in soil are enhanced through the interactions with biochar surfaces and within pores, resulting in boosted NH_4^+-N oxidation efficiency and increased AOA and AOB copy numbers (Joseph et al., 2010; Thies et al., 2015).

Nitrifiers are very sensitive to soil pH (De Boer and Kowalchuk, 2001; Sahrawat, 2008). Nitrification halts when pH is lower than 5.0, and occurs rapidly when pH is over 6.0 (Sahrawat, 2008). Most biochars are alkaline and have a liming effect on acidic soils (Brandstaka et al., 2010; Cayuela et al., 2013b). The main forms of alkalinity in biochars include oxygen-containing organic functional groups on their surfaces, mineral deposits such as $CaCO_3$, and soluble base cations that are formed during pyrolysis or inherited from feedstocks (Singh et al., 2010; van Zwieten et al., 2010a; Yuan et al., 2011). Studies on acidic soils with alkaline biochar treatment demonstrated increased nitrification rates in soil (Clough et al., 2010; Ulyett et al., 2014; Zhao et al., 2014). However, the effects of biochar on nitrification in alkaline soils remain unanswered (Cayuela et al., 2013b).

Substrate (NH_4^+-N) abundance and availability also affect nitrification; therefore inorganic NH_4-based or organic fertiliser combined with biochar creates favourable conditions for nitrifying activities (Sahrawat, 2008; Prommer et al., 2014). Adding inorganic fertiliser may stimulate nitrification due to increase substrate availability, and organic fertiliser can stimulate nitrification through increased organic matter (Song et al., 2014; Zhao et al., 2014).

Nitrification inhibitors in some biochars cause nitrification reduction (Clough et al., 2010). Biochar may contain bactericidal and fungicidal compounds such as acetaldehyde, aldehydes, α- and β-pinene, pinecarveol, and ethylene which limit microbial activities in general (Simpson and McQuilkin, 1976; Painter, 1998; Kurose et al., 2007; Clough et al., 2010; Spokas et al., 2010). The content of volatile organic compounds (VOCs) in biochar determines the bactericidal ability of biochar and depends on pyrolysis temperature and feedstock type (Clough et al., 2010). For example α-pinene, was still retained in biochar made from *Pinus* after thermal decomposition and it restricted nitrifiers i.e. *Nitrosomonas* (Ward et al., 1997). The extent in which biochar VOCs effects on nitrification and possible NO_3^--N availability remains unanswered in published studies. However, the effects of bactericidal compounds are likely to be transient and minimal since their lifetime in soil is short (Zackrisson et al., 1996; Jeffery et al., 2015).

2.2.4. Denitrification

Denitrification decreases NO_3^--N availability and is affected by biochar amendment. Biochar significantly decreases N_2O by affecting denitrification processes (van Zwieten et al., 2009; Cayuela et al., 2013b; Clough et al., 2013; Thies et al., 2015). Generally, biochar can reduce N_2O emissions by up to 50% through four possible mechanisms (Cayuela et al., 2013b).

(i) N_2O can bind with functional groups on the biochar surface, particularly metal ions embedded in biochar (e.g. Fe or Cu), and may be activated for N—N or N—O scission, leading to N_2O emission reduction (Cayuela et al., 2013b).
(ii) The substrate for microbes decreases through absorbing organic C or inorganic N. Biochar may limit the availability of N in soil, reducing substrates that produce N_2O (Cayuela et al., 2013b). In some cases, biochar reduces denitrifiers because it adsorbs SOMs on its surface and incorporates them in organo-mineral fractions, thus reducing C sources for soil microbial activities (Joseph et al., 2010).
(iii) Microbial functional groups can change due to pH changes. The liming effect of biochar helps to mitigate N_2O emission during denitrification because elevated pH reduces denitrification of NO_2^--N from nitrification and synthesis/activity of the functional N_2O reductase enzyme (Mørkved et al., 2007; Liu et al., 2010a; Bakken et al., 2012; Cayuela et al., 2013b).
(iv) Soil aeration is improved, thereby inhibiting denitrification (Heincke and Kaupenjohann, 1999).

It has also been shown that under the effects of biochar, N_2O emissions decreased whereas the abundance of nitrous oxide reductase (*nosZ*) gene increases (van Zwieten et al., 2010b; Harter et al., 2014; Bai et al., 2015a). Similarly, the abundance of *nosZ*, *nirK* and *nirS* (both nitrite reductase) genes increased with increased biochar application by up to 10% (Ducey et al., 2013). Biochar favours the last step of denitrification (i.e. converting N_2O to N_2) in which *nosZ* mediates this process; the final result is N_2O emission reduction (van Zwieten et al., 2010b; Cayuela et al., 2013a).

The crucial factors affecting N_2O emission are biochar properties (feedstock, chemical properties), biochar application rate, and N fertiliser form (Cayuela et al., 2013b). Briefly, plant-derived feedstocks, fast pyrolysis, high C:N ratio of biochar (>30), high application rates (>10%), and NO_3-based fertiliser reduced N_2O emissions the most (Cayuela et al., 2013b).

2.2.5. Nitrogen fixation

Biochar application impacts N_2-fixing bacteria (diazotrophs) including both free-living and symbiotic soil bacteria; therefore biochar influences N fixation, the conversion of atmospheric N_2 to ammonia (NH_3) (Giller, 2001; Thies et al., 2015). Free-living diazotrophs (e.g. *Azotobacter* sp. and *Azospirillum*) are stimulated in biochar treatments due to enlarged habitat with limited O_2 inside biochar pores (Thies et al., 2015). Symbionts (e.g. rhizobia) in biochar treatments are also activated. In legumes, biochar enhances biological N fixation (nodulation and nitrogenase activity) leading to increased crop yield (Clough et al., 2013; Quilliam et al., 2013; Thies et al., 2015; Xu et al., 2015). Increased application rates of biochar increases nodulation of rhizobia due to increased availability of boron (B), molybdenum (Mo), K, P, and Ca (Rondon et al., 2007). Immobilisation and adsorption of biochar with soil N reduce available N for plant roots, thus stimulating N fixation and root nodulation (Rondon et al., 2007; Biederman and Harpole, 2013; Thies et al.,

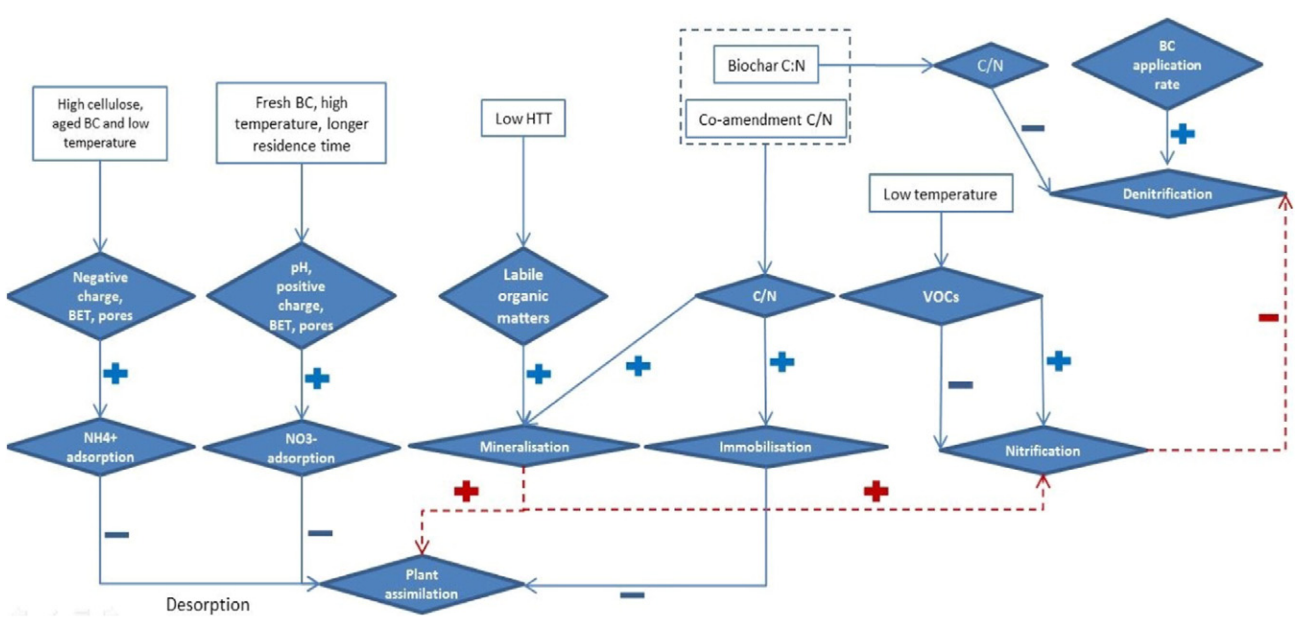

Fig. 1. Summarised effects of biochar properties on mechanisms affecting soil mineral N. The mark (+) or (−) present the increased or decreased N transformation processes.

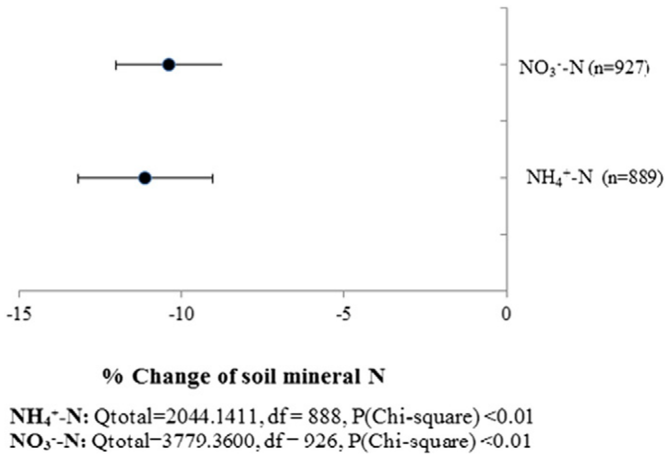

Fig. 2. Grand mean of all cases for NH_4^+-N and NO_3^--N when biochar was applied regardless of experimental conditions.

Table 2
Significance of explanatory variables by boosted regression tree (BRT) model in explaining the response of SIN under biochar effects.

	NH_4^+-N		NO_3^--N	
	Moderator	% Variation explained	Moderator	% Variation explained
1	Residence time of biochar in soil	28.1	Residence time of biochar in soil	21.7
2	Fertiliser	14.4	Biochar application rate	16.6
3	Biochar application rate	11.7	Soil pH	14.8
4	Soil pH	11.6	Pyrolysis temperature	14.2
5	Pyrolysis temperature	10	Fertiliser	11.1
6	Biochar BET	5.6	Biochar BET	5.7
7	Biochar CEC	4.4	Feedstock	5.3
8	Feedstock	4.1	Crop type	3.7
9	Pyrolysis type	3.1	Biochar CEC	2.7
10	Crop type	3.1	Pyrolysis type	2.3
11	Biochar VOCs	2	Biochar VOCs	1
12	Soil texture	1.7	Soil texture	0.7
13	Experimental type	0.2	Experimental type	0.2

2015). For example, biochar from soft wood chips reduced significantly soil N but significantly increased N fixation rate in alder (Robertson et al., 2012). In addition, biochar retains signalling molecules (e.g. nod factors) longer in soil by adsorption, and therefore increases the interaction between them and rhizobia bacteria, leading to enhanced nodulation (Thies et al., 2015). However, both short and long term studies of biochar effects on N fixation are still limited (Clough et al., 2013).

Briefly, the interaction between biochar and soil affects SIN via both abiotic and biotic pathways. However, which pathway is predominant remains unknown. For example, N immobilisation is expected to decrease SIN but if N mineralisation and nitrification are stronger, SIN is expected to increase in the soil. If SIN decreases, it means that N immobilisation and reduction processes are predominant and can be stimulated. The meta-analysis therefore may help to elucidate which pathway is stronger under certain conditions.

2.3. Other factors affecting soil inorganic N after biochar application

In the cropping system, the interaction between biochar and soil is not the only factor influencing SIN. There are other factors which may affect SIN, including the interactions between plant assimilation and fertilisation when biochar is applied.

2.3.1. Plant assimilation

Biochar alters soil NO_3^--N and NH_4^+-N availability, affecting plant assimilation. Plants can assimilate various types of N including inorganic and organic N (Schimel and Bennett, 2004; Boudsocq et al., 2012). The inorganic N preference is also different among different plant species; i.e. acid-soil plants prefer NH_4^+-N while alkaline-soil plants prefer NO_3^--N (Rosnitschek-Schimmel, 1982; Hahne and Schuch, 2004).

Biochar application improves plant uptake of SIN through different mechanisms (Chan et al., 2008) including (i) biochar improves soil moisture and pH, so it stimulates N mineralisation and nitrification leading to improved plant uptake (Saarnio et al., 2013), (ii) the liming effect of biochar creates a more favourable root zone environment by reducing the impact of soil acidity and related-toxicity (e.g. aluminium in soil could inhibit root growth) (Delhaize and Ryan, 1995; van Zwieten et al., 2010a), (iii) biochar increases N retention in soil by decreased leaching thus helping to increases inorganic N for plant

Table 1
The result of publication bias used in this study. n represents the number of the cases.

Factor	N form	Funnel plot statistics		Fail-safe numbers	5n + 1	Existence of bias	Does bias affect the trend?
		Kendall's Tau	Spearman Rank-Order Correlation				
Pyrolysis temperature	NH_4^+-N	0.02615	0.05423	43,446.2	4161	Yes	No
	NO_3^--N	0.95578	0.87989			No	No
Pyrolysis type	NH_4^+-N	0.02375	0.05102	41,113.8	4146	Yes	No
	NO_3^--N	0.92125	0.82982			No	No
Feedstock	NH_4^+-N	0.00023	0.00143	31,746.3	4386	Yes	No
	NO_3^--N	0.82701	0.76690			No	No
C:N	NH_4^+-N	0.00026	0.00160	22,430.6	3561	Yes	No
	NO_3^--N	0.00022	0.00122	23,290.8	3636	Yes	No
Application rate	NH_4^+-N	0.00049	0.00254	28,166.0	4401	Yes	No
	NO_3^--N	0.96310	0.88128			No	No
Residence time of biochar in soil	NH_4^+-N	0.00028	0.00167	31,334.8	4446	Yes	No
	NO_3^--N	0.86308	0.80047			No	No
N fertilisation	NH_4^+-N	0.00736	0.02027	19,547.8	4206	Yes	No
	NO_3^--N	0.53413	0.52056			No	No
Soil texture	NH_4^+-N	0.00003	0.00031	34,582.5	4081	Yes	No
	NO_3^--N	0.56463	0.66781			No	No
Soil pH	NH_4^+-N	0.01217	0.03210	38,611.1	3481	Yes	No
	NO_3^--N	0.42414	0.46835			No	No
Crop type	NH_4^+-N	0.00021	0.00132	31,228.5	4411	Yes	No
	NO_3^--N	0.58754	0.57800			No	No
Pot/field-based study	NH_4^+-N	0.00024	0.00147	31,266.5	4446	Yes	No
	NO_3^--N	0.86308	0.80047			No	No
Overall effect of biochar application	NH_4^+-N	0.00024	0.00147	31,266.5	4446	Yes	No
	NO_3^--N	0.86308	0.80047			No	No

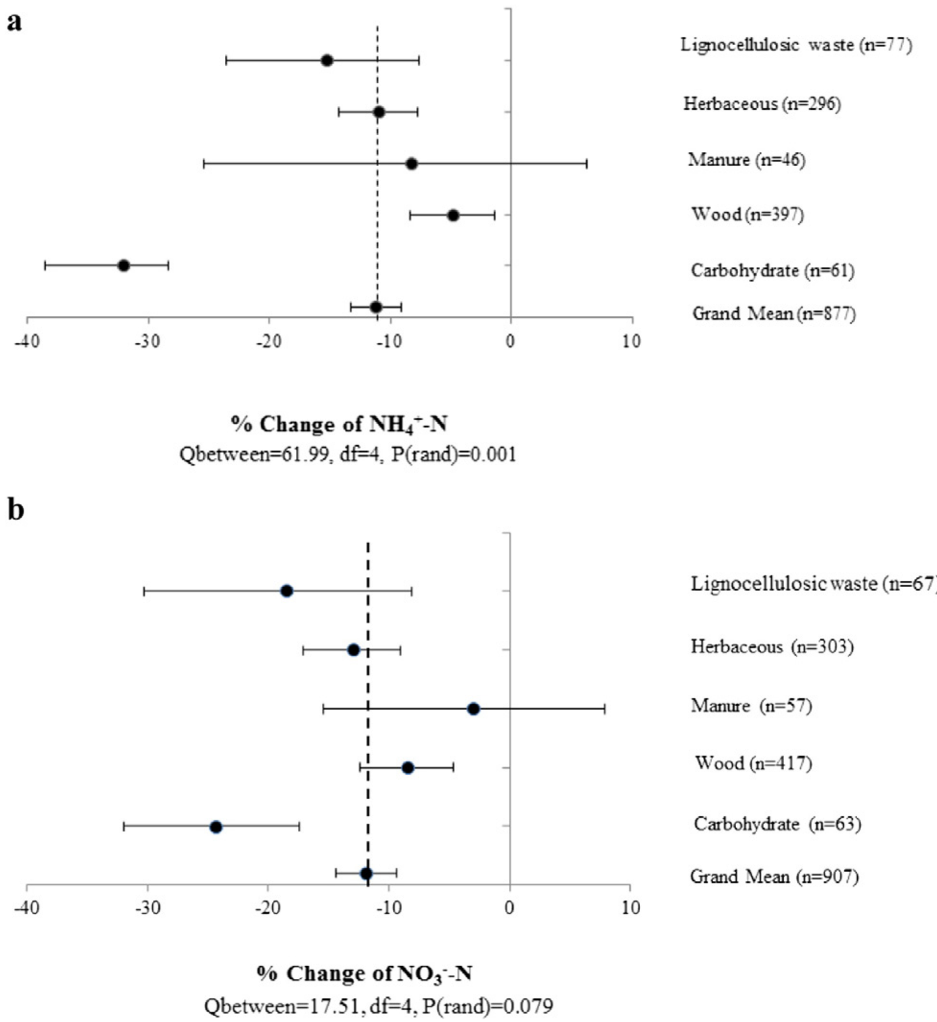

Fig. 3. Influence of different feedstock used to produce biochar on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all feedstocks when biochar is applied to soil.

assimilation (van Zwieten et al., 2010a). However, some studies did not observe any effect of biochar alone on N uptake, but observed a positive effect on N uptake when biochar was combined with N and Mg fertilisers (Dharmakeerthi et al., 2012). Currently we do not know how much plants affect SIN availability after biochar application.

Biochar also affects SIN indirectly through shifting root exudates, thereby influencing SOC turnover and biochar priming effects (Weng et al., 2015). Biochar also has potential to adsorb fresh root exudates (Joseph et al., 2010; Ameloot et al., 2013); additionally, microorganisms prefer the easily degradable root exudates than more recalcitrant SOC (Blagodatskaya et al., 2011). Biochar causes negative priming effects in the rhizosphere. When the ratio of root exudate C inputs to total soil C increases, rhizosphere priming effects are enhanced (Weng et al., 2015).

2.3.2. Interaction with fertilisation

N mineralisation could be stimulated when biochar is applied with organic fertiliser, glucose; cellulose and glucose; and ryegrass (Hamer et al., 2004; Nocentini et al., 2010; Luo et al., 2011; van Zwieten et al., 2013). This is due to the combined effects of available food sources for microorganisms and the habitat of the biochar surface (Chenu et al., 2001; Hamer et al., 2004). Bruun et al. (2011) observed N immobilisation in soil when applying biochar alone, but no N immobilisation was found when biochar was applied with slurry (C:N of 2.2). When applying an inorganic substrate, the combination of inorganic fertiliser with biochar can offset the decrease of inorganic N due to N immobilisation, thus reducing N deficiency of plants (Prommer et al., 2014). However, to date it is unknown which fertiliser addition can be more beneficial to alleviate SIN reduction by biochar.

3. Current knowledge: A quantitative analysis of the factors influencing the impact of biochar on soil inorganic N

There have been many studies which show the effects of biochar on soil N availability (See Fig. 1); however, results of these studies were contradictory. To the best knowledge of the authors, there is no meta-analysis to assess the effects of biochar on SIN to date. This meta-analysis aimed to answer the following questions (i) how biochar properties affect SIN and (ii) how the interactions between biochar properties and other factors (e.g. soil properties, fertiliser and crop type etc.) alter SIN. Based on the widely reported effects of biochar on SIN, we hypothesised that both biochar and soil properties would affect SIN due to changing soil biochemical properties after adding biochar to the soil.

3.1. Methods

3.1.1. Data sources and compilation

A literature search was undertaken of Google Scholar, USC Electronic Library, and Science Direct using keywords (biochar OR black carbon OR char OR hydrochar) AND (nitrogen OR N OR nitrate OR ammonium OR

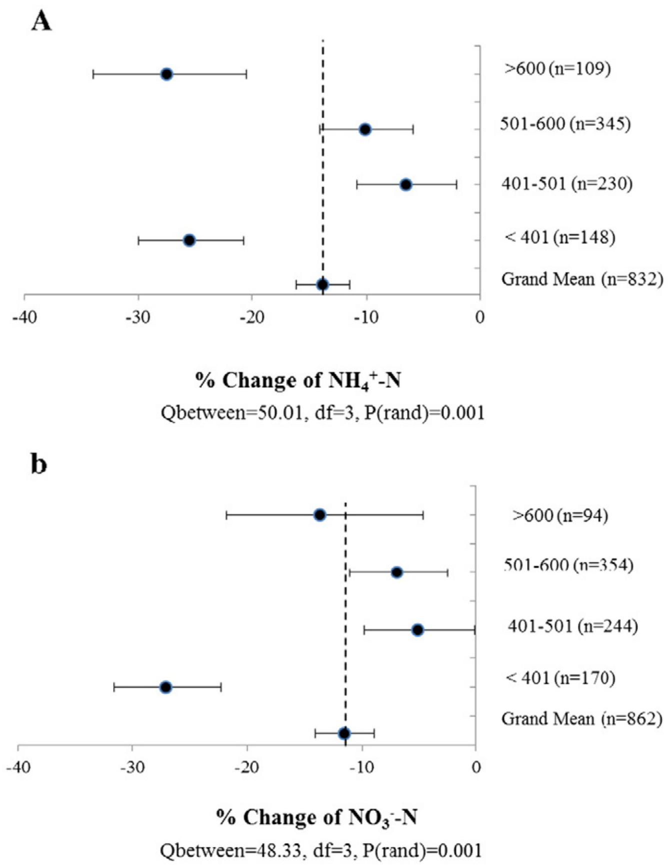

Fig. 4. Influence of pyrolysis temperature used to produce biochar on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all ranges of temperature when biochar is applied to soil.

mineral N OR inorganic N) AND (soil) NOT (review) NOT (meta-analysis). Publications were included in the meta-analysis if they met the following criteria (1) at least 3 replicates per treatment, (2) the original data on NH_4^+-N and NO_3^--N content could be extracted from the manuscript (from tables and figures) including the mean and standard deviation (SD) or standard error (SE) of the mean; and (3) data collection was carried out based on paired observation between a control and a treatment with biochar. The control was subject to the same experimental conditions without a biochar treatment.

To minimise the effect of publication bias, some unpublished studies were included. We found 55 peer-reviewed manuscripts and 1 unpublished manuscript that met the criteria published between 01/2010 and 12/2015. The process was summarised in a PRISMA statement (Moher et al., 2009) (Fig. S1).

The data on biochar characteristics, soil properties, and experimental conditions were extracted from each study, including (1) biochar characteristics: feedstock, pH, pyrolysis temperature, type of pyrolysis, C:N ratio, volatile organic matters (VOCs), %C, %N, cation exchange capacity (CEC), BET surface area; (2) soil properties: soil texture, pH, bulk density, %C, %N; and (3) experimental conditions: pot-based or field-based experiment, crop types, biochar application rate, residence time of biochar in soil, N amendments (e.g. type of amendment, application rate, %C, %N). The software Plot Digitizer 2.6.2 (Huwaldt, 2012) was used to extract data from figures. The data extracted was the mean of the control, the mean of the treatment, and standard deviation (SD) or standard error (SE). Where necessary, the corresponding authors were contacted to supply additional information.

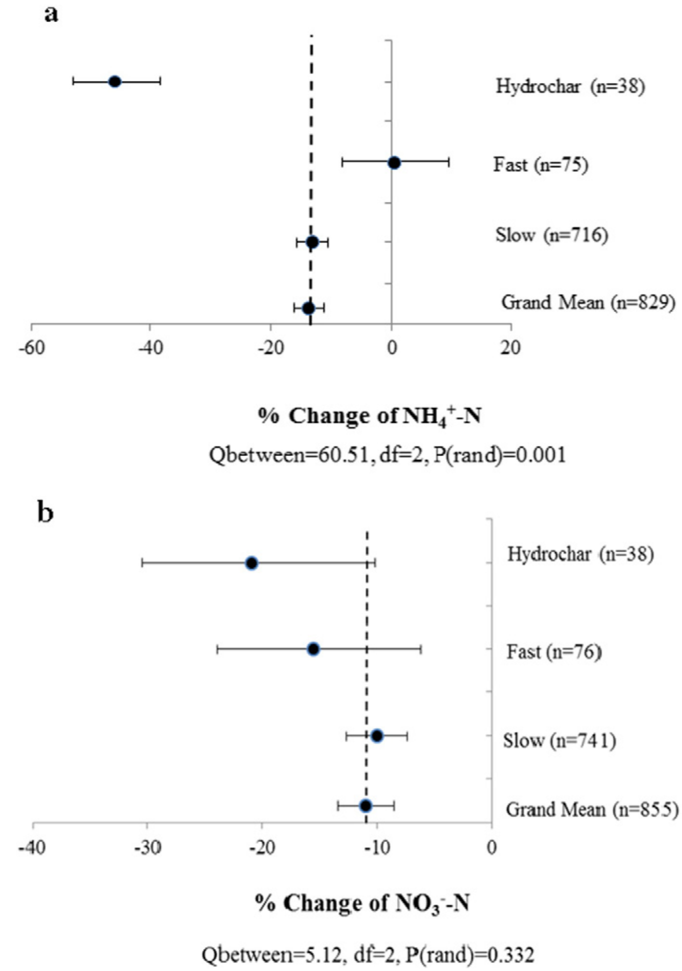

Fig. 5. Influence of pyrolysis type used to produce biochar on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all pyrolysis types when biochar is applied to soil.

The data were converted to the same units for comparison. Biochar application rate was converted from t ha^{-1} to % by using bulk density (BD) of soil and soil depth to which biochar was applied. If BD was not reported, the Hydraulic Properties Calculator program was used to determine BD based on soil texture (Saxton and Rawls, 2009). pH($CaCl_2$) and pH(KCl) were converted to pH(H_2O) by using the formulas:

$$pH(H_2O) = 1.65 + 0.86 * pH(CaCl_2)\ (r^2 = 0.70; p<0.001) \quad (1)$$

(Augusto et al., 2006)

$$pH(H_2O) = 1.96 + 0.74 * pH(KCl)\ (r^2 = 0,57; p<0,001) \quad (2)$$

(Augusto et al., 2006)

3.1.2. Effect size

The effect size was calculated by using the natural log-transformed response ratio (RR)

$$\ln RR = \ln\left(\frac{X_T}{X_C}\right) \quad (3)$$

where: X_T is the mean of the biochar treatment and X_C is the mean of the control group.

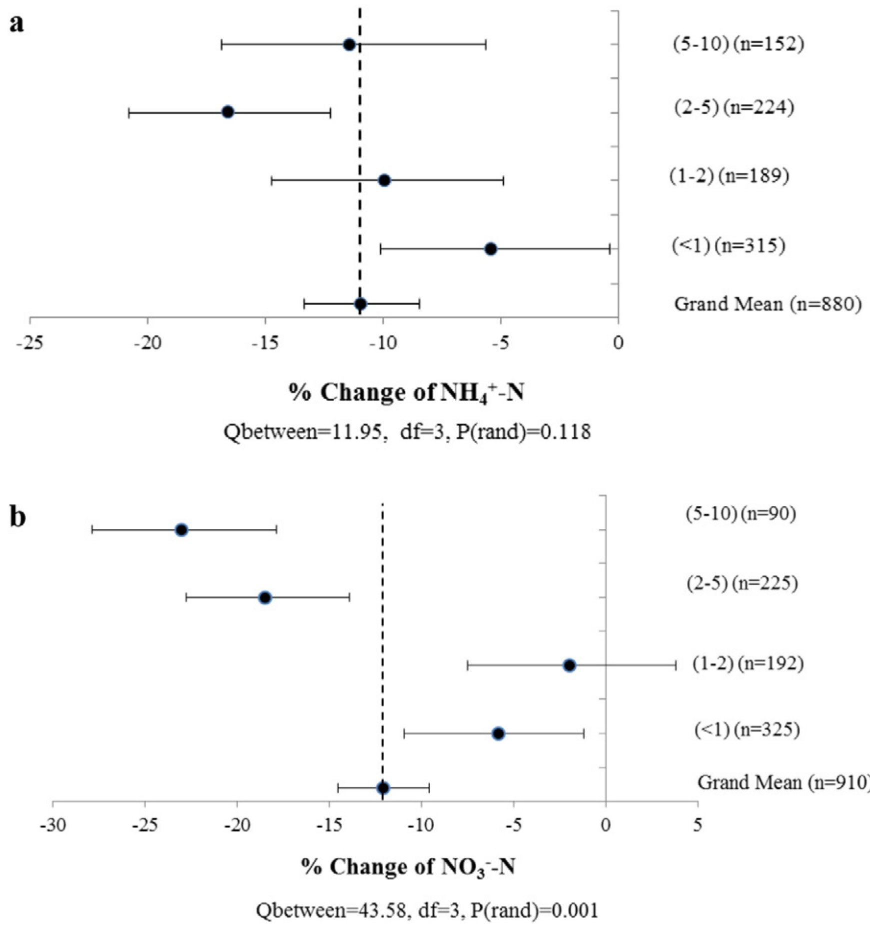

Fig. 6. Influence of application rate (% weight) on changes on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all application rates when biochar is applied to soil.

All subsequent statistical analyses below used lnRR as the response variable.

3.1.3. Mixed-effect model

A random-effect model was selected for the meta-analysis except where the estimated pooled variance was ≤0, in which a fixed effects model was used. The means and SD of the control and treatment of each study were recorded (as NH_4^+-N and NO_3^--N content). The variance was calculated by using the SD or SE values.

The data of explanatory variables were then grouped in the following categories:

- Feedstock was grouped in five categories: (1) carbohydrates/non-lignocellulosic waste (fruit peels, beet-root chips, spent brewer's grains), (2) wood, (3) manure, (4) herbaceous waste (green waste, straws, and corn stover), and (5) lignocellulosic waste (walnut shells, peanut shells, maize cobs, furfural from corn cobs) (Rosillo-Calle et al., 2015).

- Pyrolysis temperature was grouped in four categories: (1) ≤400, (2) 401–501, (3) 501–600, and (4) >600 (Cayuela et al., 2013b)
- Pyrolysis condition was grouped in three categories: (1) fast, (2) slow, (3) hydrochar (Cayuela et al., 2013b)
- The residence time of biochar in soil was divided into different subgroups including ≤1 week (<8 days), ≤1 month (<31 days), 3 months (<91 days), 6 months (<181 days), ≤1 year (<366 days), >1 year (>366) (Cayuela et al., 2013b).
- N fertilisation with biochar was grouped in five categories: (1) organic (slurry, manure, green-waste compost, and urine), (2) urea, (3) ammonium nitrate (NH_4NO_3), (4) NO_3-based, and (5) NH_4-based.
- Soil texture was grouped in three categories: (1) coarse (sandy loam, sandy clay loam, loamy sand), (2) medium (clay loam, loam, silty clay loam, silt, silt loam) or (3) fine (clay, silt clay, sandy clay) (Cayuela et al., 2013b)
- Soil pH was grouped in three categories: (1) Very acidic (pH < 5), (2) Acidic (5 < pH < 6), and (3) Neutral (pH > 6) (Jeffery et al., 2011).
- Crop types were grouped in five categories: (1) dried crop (maize, wheat, spring barley, lettuce, macadamia, and plantain), (2) grass (ryegrass, and fescue), (3) legumes (clover, bean, and peanut), (4) paddy rice, and (5) no crop.

The effect sizes of each group were calculated using a categorical random effects model, where the effect size was weighted in inverse proportion to its variance (Adams et al., 1997). Mean effect sizes of each category and the 95% confidence intervals (CIs) generated by bootstrapping (999 iterations) were calculated with MetaWin Version 2 Statistical software (Rosenberg et al., 2000). A threshold was set for weight to avoid skewing of the results due to a few cases with very low variance (very high weight). We ranked the weight of each case and then plotted the weight against the rank. The threshold of weight was determined as the tipping point at which the weight started to increase dramatically against rank (Fig. S5). All cases with weight larger than the threshold were reset as the threshold value and used in the meta-analysis. The effect sizes were converted to % change by the following equation:

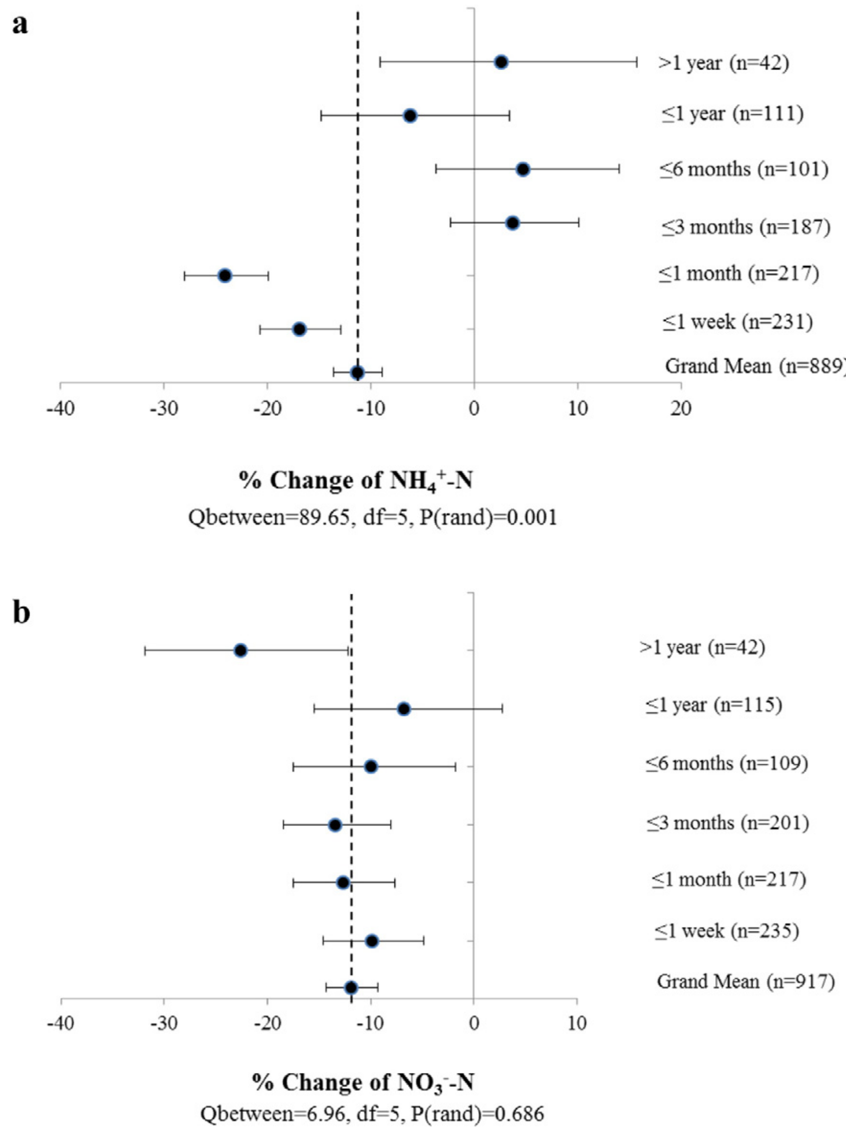

Fig. 7. Influence of time of biochar in soil on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all time ranges when biochar is applied to soil.

$$\%\text{change} = \left(e^{\ln RR} - 1\right) * 100 \quad (3)$$

(Cayuela et al., 2013b)

Mean effect sizes were considered significantly different from zero if the 95% CIs did not overlap zero, and significantly different from one another if their 95% CIs did not overlap. The mean of all effect sizes combined was calculated for NH_4^+-N and NO_3^--N.

To test the effects of publication bias (Rothstein et al., 2006) and the robustness of the meta-analysis, the funnel plot statistics and Fail-safe N technique (Rosenthal and Rosnow, 1984) were used. Fail-safe N was used only when funnel plot statistics (Kendall's Tau and Spearman Rank-Order Correlation) were significant ($p < 0.05$). The Fail-safe number was compared with 5n + 1 (n is the number of cases). To test the heterogeneity between groups, $Q_{between\ groups}/Q_{total}$ and p value of the random effect model were used when releasing the plots (Borenstein et al., 2009).

3.1.4. Boosted regression tree analysis

An additional boosted regression tree (BRT) analysis was performed to rank the importance of the explanatory variables and address non-linearity and factor interactions (Elith et al., 2008). The combination between BRT and mixed-effect model has been used in a number of meta-analyses (Rose et al., 2014; Zhang et al., 2015; Nguyen et al., 2016).

We performed a BRT analysis using the R package gbm combined with the dismo package (Ridgeway, 2013; Elith and Leathwick, 2016). A Gaussian error structure was assumed during a 10-fold cross-validation to estimate the optimal number of trees. To find the optimal setting, learning rate (0.01, 0.005, 0.001) and bagging fraction (0.5, 0.6, 0.75) were systematically altered to assess potential improvements to model fits. Among the fitted models, we found that the best model had a cross-validation deviance of 0.271 (±0.022) for NH_4^+-N and 0.248 (±0.042) for NO_3^--N from a learning rate of 0.01, bag fraction of 0.75, and 10-fold cross-validation. Tree complexity was set to 5 to address factor interactions (Elith et al., 2008).

3.2. Results

3.2.1. General trend

Our meta-analyses showed that biochar generally reduced soil mineral N by over 10% (Fig. 2). In particular NH_4^+-N was reduced by 11 ± 2% and NO_3^--N by 10 ± 1.6% regardless of experimental designs, biochar

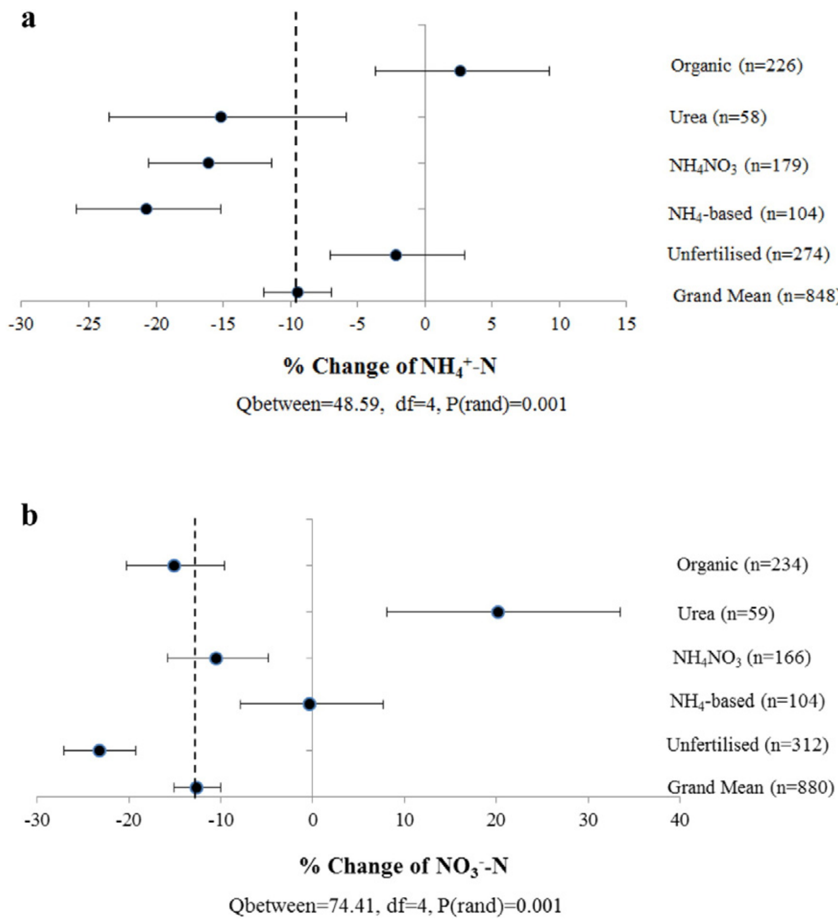

Fig. 8. Influence of N-fertiliser type on changes on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all fertilisation types when biochar is co-applied to soil.

characteristics and time since application. The interactions between biochar and environmental factors (e.g. fertilisation, soil pH, application rates, and residence time of biochar in soil) best explained SIN changes (Table 2). Among factors related to biochar properties, pyrolysis temperature explained SIN the most (Table 2).

Heterogeneity was detected in most analyses of this meta-analysis. This was inevitable because this meta-analysis included the data from a variety of experiment designs, biochar characteristics, soil properties, climate conditions, and crop types. Publication bias did not affect the trend of the plots, suggesting that the results were reliable (Table 1).

3.2.2. Influence of biochar properties on SIN

Soil NH_4^+-N was reduced the least in wood and the most in carbohydrate-based biochars (Fig. 3a). NO_3^--N was reduced more by both carbohydrate and lignocellulosic-based biochars than other feedstocks (Fig. 3b). Manure biochar interestingly did not have a significant effect on SIN.

Soil NH_4^+-N was decreased in high and low highest treatment temperature (HTT) biochars (<401 and >600 °C) (Fig. 4a). NO_3^--N was decreased by low HTT biochar (<401 °C) (Fig. 4b).

Hydrochar dramatically decreased NH_4^+-N ($-46 \pm 7\%$) whereas fast pyrolysed biochar showed no significant effect on NH_4^+-N (Fig. 5a). All biochar types significantly reduced soil NO_3^--N but there was no significant difference among types (Fig. 5b).

Only 39% of cases reported the value of biochar BET surface area, 11% of cases reported the value of biochar VOCs, and 47% of studies reported biochar CEC while methodologies for CEC determination varied between studies. The BRT model suggested that high CEC biochar reduced more soil NH_4^+-N, and high BET surface area biochar reduced more SIN (Fig. 12). At concentrations of higher than 18%, VOCs reduced more soil NO_3^--N (Fig. 12i,k).

3.2.3. Influence of the interactions between biochar and environmental factors on SIN

Between the high rate biochar applications (2-5% and 5-10%) and low rate biochar applications (<1 and 1-2%), the high rate biochar applications caused the higher reduction of NH_4^+-N but this was not the case for NO_3^--N (Fig. 6b). Most studies used an application rate < 5% while higher application rates were less common. The data on the rate >10% was very limited (six cases) and could not be used to provide a reliable conclusion.

Biochar greatly reduced NH_4^+-N one month after application ($-24 \pm 4\%$) whereas no significant change of NH_4^+-N was observed for longer residence times of biochar in soil up to 1 year (Fig. 7a). Interestingly, different time intervals did not affect NO_3^--N since there was no significant difference in NO_3^--N content during the time (Fig. 7b). However, biochar significantly reduced NO_3^--N at all time intervals except ≤1 year.

Biochar combined with urea, NH_4^+-based and NH_4NO_3 fertiliser reduced NH_4^+-N significantly, but biochar with organic fertiliser had no change on NH_4^+-N (Fig. 8a). Biochar with urea increased NO_3^--N but it decreased NO_3^--N if it was combined with NH_4NO_3 and organic-based fertilisers (Fig. 8b). Biochar alone reduced both form of SIN.

Biochar application reduced the NH_4^+-N content in coarse soils to a greater extent than in medium-textured soils (Fig. 9). Biochar also reduced more NH_4^+-N in acid groups than in the neutral group (Fig. 10a). NO_3^--N decreased with the increase of soil pH under biochar effects (Fig. 10b).

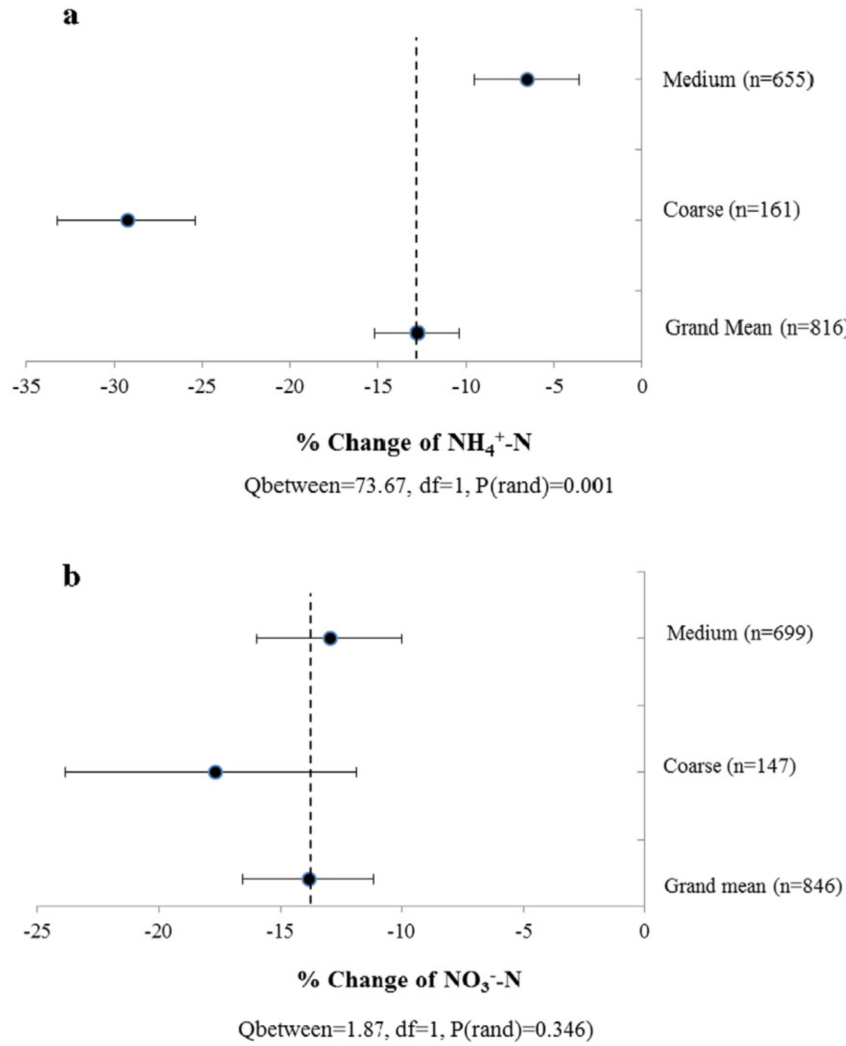

Fig. 9. Influence of soil texture on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all soil texture types when biochar is co-applied to soil.

Biochar reduced soil NH_4^+-N in dried crop or without any crop (Fig. 11a). A significant decline in NH_4^+-N was also found in pot-based experiments (Fig. S3). The effects of biochar on NO_3^--N were similar in different crop types and experimental designs (Figs. 11b, S2b). The number of paddy rice cases were limited (<10 cases), therefore paddy rice was excluded from the figures.

3.3. Discussion

The most noticeable findings are (1) the overall reduction of approximately 10% of SIN after biochar addition regardless of experimental conditions. However, the studies used to investigate biochar applications on SIN have been mainly undertaken within the first year following the biochar application (with only 42 out of 917 cases recorded after one year); (2) The interactions between biochar and environmental factors (e.g. fertilisation, soil pH, application rates, and residence time of biochar in soil), and pyrolysis temperature best explained SIN changes; (3) woody biochar did not decrease SIN as much as other biochars produced from different feedstocks; and (4) the effects of biochar on SIN were strongly affected by fertiliser type; biochar applied with NH_4-based fertilisers interestingly reduced soil NH_4^+-N the most, while biochar with organic fertiliser reduced soil NO_3^--N the most.

The overall reduction of SIN by biochar is concordant with the literature since biochar has been proven to reduce N availability as the result of high biochar C:N (N immobilisation) and high surface area (adsorption) (Lehmann et al., 2003; DeLuca et al., 2015). Two main implications of this reduction are (1) decrease of SIN leaching, and (2) reduced SIN availability for crops. Hence, SIN reduction may lead to reduced N uptake of plants in the short term after biochar application, but over the long term, available N may increase resulting in the increase of the overall crop yield (Biederman and Harpole, 2013; Bai et al., 2015a; Bai et al., 2015b). For example, there are reports of increased NO_3^--N and NH_4^+-N after 1, 2 and 10 years following biochar application (Bai et al., 2015a; Zhao et al., 2015; Hosseini Bai et al., 2016). In another study, NH_4^+-N and NO_3^--N did not differ between control and biochar (greenwaste and poultry biochar) plots, 5 years following biochar application (Bai et al., 2015b). It has also been reported that biochar is able to release immobilised N slowly over time to be assimilated by plants (Taghizadeh-Toosi et al., 2012b). This slow released N can offset the fertiliser inputs over time (Taghizadeh-Toosi et al., 2012b).

3.3.1. The effects of biochar properties on N availability

Greater reduction of SIN was observed when biochars were produced from carbohydrate, in low HTT, and hydrothermal conditions (i.e. hydrochar) compared to other biochars. Labile C content was higher in hydrochar, carbohydrate and low HTT biochar (Downie et al., 2009; Malghani et al., 2013; Singh and Cowie, 2014); also functional groups were higher in low HTT biochar than other biochars. Therefore, among

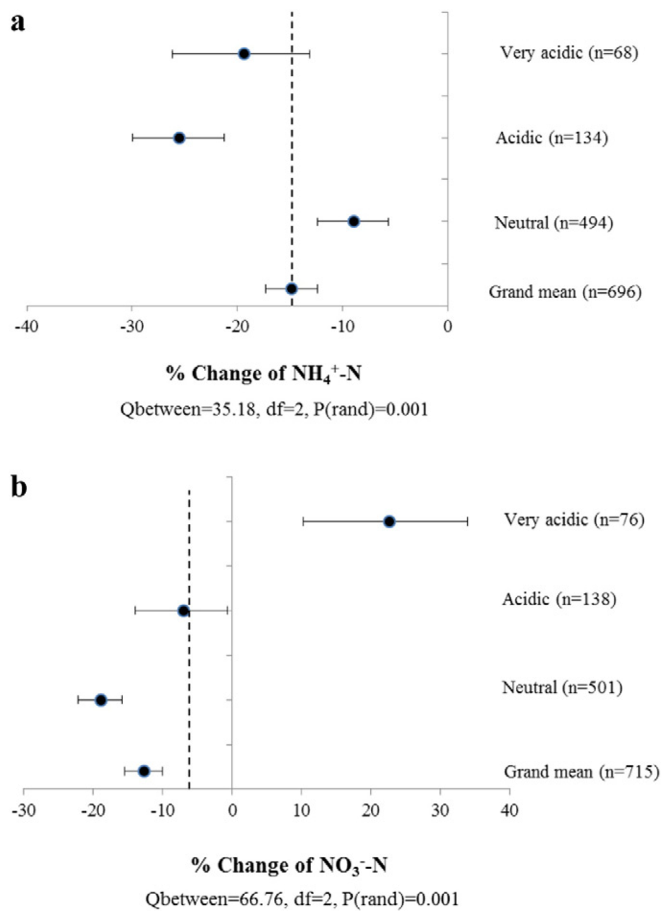

Fig. 10. Influence of soil pH on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all pH ranges when biochar is co-applied to soil.

biochar properties, pyrolysis temperature and surface properties of biochar are important factors influencing SIN.

Carbohydrates followed by lignocellulosic waste biochar decreased N the most because they had higher labile C as a food source for microbes than other biochars, leading to increased N immobilisation (Hilscher et al., 2009; Knicker, 2010). Also, the majority of carbohydrates groups were hydrochar which may have influenced on their effects. Hydrochar has lower carbonisation, higher H/C and O/C ratios, and higher concentration of easily degradable C compounds than biochar (Liu et al., 2010b; Qayyum et al., 2012; Bai et al., 2013). For example, dissolved organic C (DOC) contents of hydrochar and biochar produced at ≤500 °C from corn silage are 12.99 and 0.033 mg g^{-1}, respectively (Malghani et al., 2013); DOC of hydrochar and biochar produced at 190 °C from spent brewer's grains are 54.969 and 0.016 mg g^{-1}, respectively; from beetroot chips are 15.548 and 0.1 mg g^{-1}, respectively (Bargmann et al., 2014).

Noticeably, the least decrease of SIN was found for woody biochar as woody biochar had lower CEC, acid functional groups, and lower labile C compounds than crop-derived and herbaceous biochars, leading to lower N immobilisation and N chemisorption (Brewer et al., 2011; Harvey et al., 2012; Wang et al., 2016). Despite the fact that woody biochar had a high C:N ratio, N immobilisation rate depended on the portions of easily mineralisable C and biological recalcitrant C in biochars (Chan and Xu, 2009); whereas woody biochar contains less degradable C than other biochars (Nelissen et al., 2015; Wang et al., 2016). For example, labile C content (g kg^{-1}C) of biochars produced at 400 °C could be ranked in the order of wood biochar (2.6) < leaf biochar (6.2) < cow manure (18.3) < poultry litter (32); produced at 550 °C followed the same order of wood, leaf, cow manure, and poultry manure (1.3, 3.0, 4.9, 6.7, respectively) (Singh and Cowie, 2014). In our database, the averages CEC of herbaceous and lignocellulosic biochar (37.59 and 34.66 cmol kg^{-1}, respectively) were also higher than that of woody biochar (23.48 cmol kg^{-1}). Our BRT model also supported the significant correlation between biochar CEC and NH_4^+-N adsorption (Fig. 12).

More SIN was decreased in low HTT biochar (≤400 °C) than in other biochars. At low temperatures, acid functional groups, some labile C and bio-oils remained on the biochar surface and enhanced N adsorption and N immobilisation (Downie et al., 2009; Anderson et al., 2011; Gai et al., 2014; DeLuca et al., 2015; Whitman et al., 2015). High HTT biochar (>600 °C) reduced more NH_4^+-N by physisorption and NO_3^--N by chemisorption (Downie et al., 2009; Kameyama et al., 2012; Clough et al., 2013). Our BRT model showed significant correlation between biochar BET surface area and SIN (Fig. 12).

3.3.2. The interaction between biochar and environmental factors

Our study indicated that biochar decreased SIN when it stayed in soil for a short term (<1 month), was applied at high rate (≥2%), and in coarse-textured soil. NH_4^+-N also declined more in biochar-inorganic N fertiliser combination and acidic soil. NO_3^--N was reduced more if biochar was applied alone, with organic fertiliser, and in neutral soil.

The reduction of SIN by increased biochar application rate may be due to higher adsorption and N immobilisation. A short time after biochar application (up to one month), N immobilisation and adsorption was boosted because soil C:N ratio and biochar adsorption surface were enhanced. (Rondon et al., 2007; Downie et al., 2009; Hammes and Schmidt, 2009; Xu et al., 2015). Multicollinearity was detected between biochar rate, feedstock type, pyrolysis temperature, and biochar BET surface area moderating NO_3^--N (Tables 3, S2). Most biochars in the high application rate groups were produced from high carbohydrate feedstocks, with low HTT and a high BET area which explained NO_3^--N reductions (Figs. 3b, 4b, 12). This multicollinearity contributed to a greater NO_3^--N reduction in biochar applied at high rates. In contrast, low rate groups contained 89% cases with manure biochars, high HTT and low BET area which did not result in decreased NO_3^--N. NO_3^--N reduction depended on both the biochar application rate and also the feedstock type and pyrolysis temperature. Such multicollinearity was not observed for NH_4^+-N.

Over longer residence times, N immobilisation was reduced due to decreased labile organic compounds and adsorption capacities of biochar when organic and mineral compounds built up on biochar surface (Pignatello et al., 2006; Cheng et al., 2008; McLaughlin et al., 2009; Joseph et al., 2010; Singh and Cowie, 2014). A negative priming effect (decrease of mineralisation) was also observed in the long term (>250 days) (Zimmerman et al., 2011). The long-term reduction of NO_3^--N might be due to leaching but the mechanism remained unclear (Ventura et al., 2013). Long-term effects of biochar were a shortcoming of this meta-analysis because the studies of long-term biochar were limited (Table 4).

Our study suggested that fertiliser type added to biochar is also a crucial factor affecting SIN. We found that (1) adding organic fertiliser to biochar only reduced NO_3^--N; (2) adding urea and NH_4NO_3 fertiliser to biochar increased and decreased NO_3^--N respectively, (3) adding inorganic N fertilisers to biochar reduced soil NH_4^+-N, and (4) without fertiliser, biochar significantly reduced NO_3^--N. CEC in biochar-amended soil was enhanced when organic fertiliser was co-applied because organic fertiliser also contributed to increase SOM; so more soil NH_4^+-N was adsorbed and the NH_4^+-N pool for nitrification declined (Ulyett et al., 2014). Our result was contradictory with van Zwieten et al. (2013) who observed increased NO_3^--N after poultry litter biochar and organic amendment addition because organic amendments (poultry litter) promoted N mineralisation within a short period of time following biochar application (7–35 days), leading to an increase in SIN (van Zwieten et al., 2013). Biochar alone (unfertilised) significantly

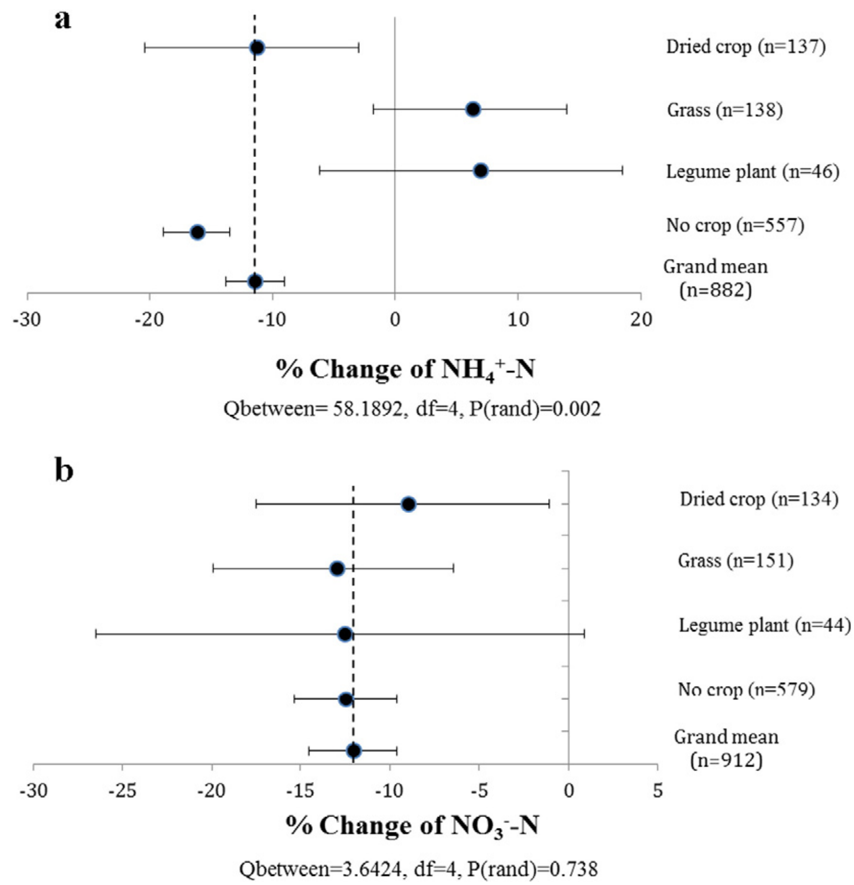

Fig. 11. Influence of crop type on NH_4^+-N and NO_3^--N availability in soil. Symbols represent mean effect sizes (percentage of change in NH_4^+-N (a) and NO_3^--N content (b)) with 95% confidence intervals. The numbers shown on the right correspond to observations in each class upon which the statistical analysis is based (n). The dotted line indicates the mean effect size for all pH ranges when biochar is co-applied to soil.

reduced NO_3^--N possibly due to adsorption and immobilisation (please see Sections 2.1, 2.2.2). Also, it should be noted that most biochars in the unfertilised group were applied at high rates (≥2%) and in neutral soil, which reduced NO_3^--N the most (Figs. 6b, 10b). Therefore, this multicollinearity contributed to extending NO_3^--N reduction in unfertilised groups in our database. NO_3^--N increase by biochar and urea combinations may be associated with nitrification stimulation due to increased substrates for nitrifiers (van Zwieten et al., 2013; Shen et al., 2014). Biochar co-applied with NH_4NO_3 and NH_4^+-based fertiliser did not show the obvious effect on soil NO_3^--N as much as urea because the application rate of urea was higher than that of NH_4NO_3 and NH_4^+-based fertiliser. Alburquerque et al. (2013) also found that adding NH_4NO_3 fertilisation to biochar increased soil NO_3^--N at high rates compared to low rates (58 vs 144 mL per pot) when fertiliser was applied in biochar rates of ≤1%, suggesting that the high rate of fertiliser may be necessary to stimulate nitrification significantly when it is combined with biochar (Alburquerque et al., 2013).

In the presence of inorganic fertiliser, biochar reduced NH_4^+-N, perhaps due to adsorption equilibrium and nitrification (Bohn et al., 2002), as supported by the BRT model that fertiliser type interacted with biochar CEC and soil pH. The multicollinearity between type of fertiliser, biochar feedstock and residence time of biochar in soil might mask the effect of biochar alone on soil NH_4^+-N. Most of the cases in our unfertilised group used manure biochar and stayed in soil for more than one month, which had no effect on NH_4^+-N (Figs. 3a, 7a).

Our study indicated that biochar application decreased NH_4^+-N in coarse soils more than in other soil texture types, but soil texture did not moderate the effects of biochar on soil NO_3^--N. This is possibly attributed to volatilisation of NH_3 because 90% of cases in the coarse group had acidic soil pH, whereas biochar increased NH_3 volatilisation by elevating soil pH (Clough et al., 2013). The BRT model supported this by showing the interaction between soil pH and soil texture (Table S1). Although the literature has shown that biochar could reduce NH_3 emission if biochar is combined with organic fertiliser or during composting (Clough et al., 2013), our meta-analysis did not include any case of biochar-organic fertilisation within the coarse group due to the lack of data.

Our results indicated that biochar only increased NO_3^--N significantly in very acidic soils (pH < 5) probably by increased nitrification and decreased denitrification due to the biochar liming effect (Cayuela et al., 2013b). Adding biochar to acidic or very acidic soil has been shown to have a greater liming effects in soil leading to affect SIN compared to those applied in neutral or calcareous soil where the activity of nitrifiers is high and nitrification inhibitors are naturally lacking (Lehmann et al., 2003; Rondon et al., 2007; Clough and Condron, 2010; Zhao et al., 2014). Moreover, as shown by the BRT model, biochar reduced NO_3^--N the most in neutral soil because there was multicollinearity in our meta-analysis between soil pH, pyrolysis temperature and biochar rate (Table 3, S2). 67% and 73% of the cases in the neutral group were biochar produced at ≤400 °C and applied at ≥2%, respectively, which have been shown to reduce the most NO_3^--N (Figs. 4b, 6b). Regarding nitrification, our BRT model also confirmed that biochar with high concentration of VOCs reduced more soil NO_3^--N since VOCs have been reported to be nitrifier inhibitors (please see Section 2.2.3).

Biochar significantly reduced SIN in both unplanted and planted systems with an exception observed for legumes (Fig. 11). The negative effects of plants on SIN were perhaps due to increased uptake of SIN by plants when biochars were applied (Chan et al., 2008). It was likely that no effect of biochar on NH_4^+-N in grass was associated with the interaction between crop type, fertiliser, and pyrolysis temperature in our

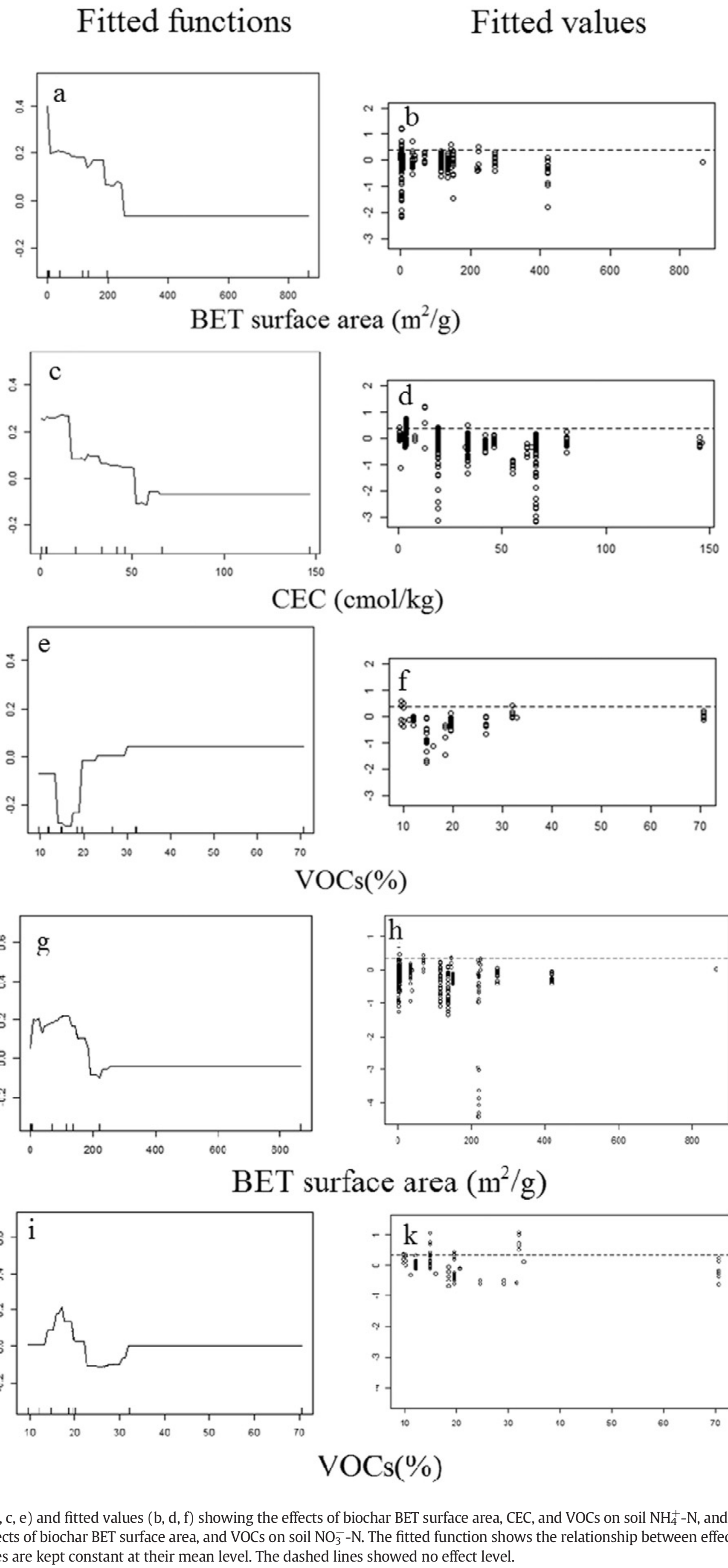

Fig. 12. Partial dependence plots (a, c, e) and fitted values (b, d, f) showing the effects of biochar BET surface area, CEC, and VOCs on soil NH_4^+-N, and partial dependence plots (g, i) and fitted values (h, k) showing the effects of biochar BET surface area, and VOCs on soil NO_3^--N. The fitted function shows the relationship between effect sizes and an explanatory variable while all other explanatory variables are kept constant at their mean level. The dashed lines showed no effect level.

Table 3
The 8 most important pairwise interactions by boosted regression tree (BRT) model to address the factor interactions.

	NH_4^+-N			NO_3^--N		
	Factor 1	Factor 2	Interaction size	Factor 1	Factor 2	Interaction size
1	Fertiliser	Biochar application rate	5.63	Biochar application rate	Pyrolysis temperature	35.17
2	Soil pH	Biochar CEC	5.12	Soil pH	Biochar application rate	13.93
3	Soil pH	Biochar application rate	4.19	Biochar application rate	Biochar BET surface area	10.74
4	Fertiliser	Residence time of biochar in soil	3.47	Biochar application rate	Feedstock	8.84
5	Fertiliser	Biochar CEC	2.46	Fertiliser	Biochar application rate	8.55
6	Fertiliser	Soil pH	2.13	Soil pH	Pyrolysis temperature	3.14
7	Fertiliser	Feedstock	2.05	Residence time of biochar in soil	Pyrolysis temperature	1.80
8	Crop Type	Soil pH	1.67	Residence time of biochar in soil	Biochar application rate	1.56

meta-analysis data. Biochars in 83% and 72% cases of grass group were applied with organic fertiliser and produced at mid-range temperature, respectively, which had no effect or little negative effect on soil NH_4^+-N (Figs. 4a, 8a). No effect by legumes on SIN was possibly because the positive effects of biochar on SIN by fixation (Clough et al., 2013; Quilliam et al., 2013; Thies et al., 2015; Xu et al., 2015) hindered the negative effects of biochar on SIN. Legumes contributed to increased N in soil leading to confounded effects of decreased SIN after biochar application. In unplanted systems, however, SIN was reduced mainly by adsorption and immobilisation as reported by the literature in our meta-analysis (Zavalloni et al., 2011; Zheng et al., 2012; Ducey et al., 2013), and volatilisation when soil pH was increased by the biochar liming effect (Bargmann et al., 2014).

The effects of biochar on soil NH_4^+-N in pot-based trials can overestimate the field-based results as suggested by different meta-analyses (Jeffery et al., 2011; Liu et al., 2013). We also found a strong confounding effect between pot and field experiments. In our meta-analysis database, 83% of field trials focused on longer term effects of biochar (>1 month) in soil, while 69% of pot trials investigated short term effects (≤1 month). Therefore, it is not possible to conclude NH_4^+-N was reduced more in pot trials on the basis of this analysis. Further studies are needed to test the combined effects of experimental design and residence time of biochar in soil.

4. Conclusion and perspective

This meta-analysis showed an overall negative, statistically significant but moderate effects of biochar application on SIN (approximately $-11 \pm 2\%$ of NH_4^+-N and $-10 \pm 1.6\%$ NO_3^--N). The N cycling in soils amended with biochar is potentially complex because of the diverse properties of biochars and soils as well as the interactions between them. This highlights the need for further long-term experiments that quantify the effects of factor combination (e.g. fertiliser type and biochar application rate, soil pH and biochar application rate, feedstock type and biochar application rate, etc.) on SIN.

Some other recommendations need to be considered when applying biochar into soil (i) woody biochar can be applied for less reduction of N in soil; (ii) carbohydrate biochar should be combined with organic fertiliser to offset the decreases of SIN; (iii) biochar should be applied at least one month prior to planting to the soil so plants do not suffer decreased N within the first month; and (iv) hydrochar and high rate biochar application could be combined with organic fertiliser to offset the effect of N immobilisation via stimulation of N mineralisation. Recently developed enhanced (organo-mineral) biochar, a low-dose, high-efficiency biochar–fertiliser product, may also offset the reduction caused by conventional biochar application (Joseph et al., 2013; Darby et al., 2016; Ye et al., 2016). Enhanced biochar is produced by torrefaction of the mixture between biochar and other materials (e.g. clay, ground rock, minerals); pre-pyrolysis (i.e. slow-pyrolysing feedstock with minerals and nutrients at low temperature 350–450 °C), post-pyrolysis (i.e. mixture of biochar and minerals/nutrients/manures is treated with heat or chemicals), and composting biochar with organic materials (Joseph et al., 2013; Lin et al., 2013). Application of enhanced biochar to soils can reduce the fertiliser input, be a better slow-released fertiliser for plants than other biochar products, increase disease resistance, germination rates, and nutrient uptake of plants, and reduce N leaching (Joseph et al., 2013; Lin et al., 2013; Chia et al., 2014; Darby et al., 2016).

This meta-analysis identified factors influencing SIN after biochar application including residence time of biochar in soil, fertilisation, soil properties (texture and pH), biochar application rate, feedstock, temperature and type of pyrolysis, BET surface, CEC, and VOCs content of biochar. However, the influence of other potentially crucial factors could not be considered due to a lack of auxiliary data including pyrolysis time interval of biochar production, biochar activation type, electrical conductivity (EC) of biochar, the size of biochar particles in biochar treatments, C:N ratio of organic fertiliser combined with biochar, and SOM content. This issue led to reduction of the number of studies that could be included in this analysis. Long-term studies (>5 years) have not been tested in our met-analysis due to limited number of studies. These shortcomings of this meta-analysis were summarised in Table 3.

In brief, the future studies on biochar and SIN should focus on a mechanistic understanding of the interactions of biochar in soil. Some basic information should be reported when a study is published including (i) production conditions (activation type, atmospheric pressure, feedstock properties e.g. origin, elemental analysis), (ii) biochar properties (CEC, surface area, particle size, EC, adsorption capacity), and (iii) environmental conditions and soil management (meteorological data, tillage, cultivation, crop type).

Table 4
Research gaps in the current knowledge on the effects of biochar on soil inorganic N.

Gap	Description
Production condition	Data should be fully reported including pyrolysis time interval of biochar production, activation type, atmospheric pressure, feedstock properties e.g. origin, elemental analysis
Biochar properties	Data should be fully reported including surface area, CEC, VOCs, particle size, EC, adsorption capacity, and the size of biochar particles
Biochar application rate	Very limited studies on high rate (>10%)
Residence time of biochar in soil	Very limited studies on long term (>5 years)
N fertiliser type	C:N ratio of organic fertiliser combined with biochar should be reported
Environmental conditions and soil management	Data should be fully reported including meteorological data, tillage, cultivation, soil organic matter, crop type
Effects of factor combination which influences biochar effects on SIN	Long-term experiments that quantify the effects of factor combination (e.g. fertiliser type and biochar application rate, soil pH and biochar application rate, feedstock type and biochar application rate, etc.) on SIN.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2016.11.004.

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