GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2012) **4**, 751–760, doi: 10.1111/j.1757-1707.2012.01163.x

'Bioenergy from cattle manure? Implications of anaerobic digestion and subsequent pyrolysis for carbon and nitrogen dynamics in soil'

SOCRATES SCHOUTEN*, JAN W. VAN GROENIGEN*, OENE OENEMA*† and MARIA L. CAYUELA*‡

**Department of Soil Quality, Wageningen University, PO Box 47, 6700 AA Wageningen,The Netherlands, †Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands, ‡Department of Soil and Water Conservation and Waste Management, CEBAS, CSIC, PO Box 164, 30100 Espinardo, Murcia, Spain*

Abstract

Cattle manure can be processed to produce bioenergy, resulting in by-products with different physicochemical characteristics. To evaluate whether application of such bioenergy by-products to soils would be beneficial compared with their unprocessed counterpart, we quantified differences in greenhouse gas emissions and carbon (C) and nitrogen (N) dynamics in soil. Three by-products (^{15}N-labeled cattle manure, from which anaerobic digestate was obtained, which was subsequently pyrolysed) were applied to a loess and a sandy soil in a laboratory incubation study. The highest losses of soil C from biological activity (CO_2 respiration) were observed in manure treatments (39% and 32% for loess and sandy soil), followed by digestate (31% and and 18%), and biochar (15% and and 7%). Emissions of nitrous oxide (N_2O) ranged from 0.6% of applied N from biochar to 4.0% from manure. Isotope labeling indicated that manure N was most readily mineralized, contributing 50% to soil inorganic N. The anaerobic digestate was the only by-product increasing the mineral N pool, while reducing emissions of N_2O compared with manure. In biochar treatments, less than 18.3% of soil mineral N derived from the biochar, while it did not constrain mineralization of native soil N. By-products of anaerobic digestion and pyrolysis revealed soil fertility in addition to environmental benefits. However, the reported advantages lessen when the declining yields of C and N over the bioenergy chain are considered.

Keywords: ^{15}Nitrogen, biochar, bioenergy, digestate, manure, soil

Received 5 November 2011 and accepted 6 December 2011

Introduction

Recent concerns that bioenergy production from conventional crops might negatively affect food production has shifted research toward the development of 'second generation' biofuels from alternative sources of biomass (Koh & Ghazoul, 2008). A potentially large contribution could come from crop residues and animal manures (Woolf *et al.*, 2010). However, crop residues and manures fulfill an important role in the maintenance of soil quality. Lal (2005) estimated that the removal of 30 to 40% of crop residues from land can deplete the soil organic matter (SOM) pool and cause land productivity to decline. However, a mature bioenergy industry will generate considerable quantities of bioenergy by-products, which might be returned to the soil instead (Cayuela *et al.*, 2010; Taheripour *et al.*, 2010). A shift in products used as soil amendments will translate into effects on greenhouse gas (GHG) emissions, carbon storage, and soil fertility, which should be included in the overall assessments of bioenergy chains.

Animal manures may be processed to improve their properties and to derive energy. For example, they may be physically separated in a liquid and a solid fraction, or be subject to anaerobic digestion (Bertora *et al.*, 2008). The digestion process decreases the feedstock's carbon : nitrogen ratio and chemical and biological oxygen demand, while yielding biogas, a mixture of methane (CH_4), carbon dioxide (CO_2) and trace gases (Bousfield *et al.*, 1979; Ward *et al.*, 2008). Most research suggests that especially the easily mineralizable carbon (C) and nitrogen (N) are digested, resulting in lower emissions of GHGs, such as CO_2 and nitrous oxide (N_2O) and greater stability of C in the soil following their application (Amon *et al.*, 2006; Marcato *et al.*, 2009; Möller & Stinner, 2009), although other workers found no differences (Clemens *et al.*, 2006; Bertora *et al.*, 2008).

Correspondence: Maria Luz Cayuela, tel. + 34 968 39 6109, fax + 34 968 39 6213, e-mails: mlcayuela@cebas.csic.es; marialuz.cayuela@gmail.com

Another bioenergy platform that currently gains much attention is pyrolysis (Laird *et al.*, 2009). Its solid by-product, 'biochar', is rich in stable aromatic compounds and has been identified as means to enhance soil fertility and sequester photosynthetically fixed C in soils (Lehmann *et al.*, 2006; Gaunt & Lehmann, 2008). The N present in biochars is also predominantly present in persistent, heterocyclic compounds (Knicker, 2010). Recent studies indicate that soils amended with biochar may even reduce soil N_2O emissions, most likely by affecting the rates of nitrification and denitrification (Clough & Condron, 2010; Zhang *et al.*, 2010). Hence, there are multiple treatment or conversion options to change the nature of C and N in biomass residues and the nature and rate of soil processes following their application to soil. Especially when applied in sequence, the processing steps increasingly derive energy from the residue and change its composition. Recent work suggests that pyrolysis of anaerobically digested matter results in properties beneficial for soil application (Inyang *et al.*, 2010). However, such a sequence also increasingly reduces the total and easily degradable C and N content of the residue. We question whether these modifications encourage the use of bioenergy by-products as soil amendments; their impact on SOM quality and nutrient availability remain largely unknown.

The comparison of different residues in terms of C and N dynamics may be assisted by stable isotope techniques. Stable isotopes may be used as biomarkers to follow the evolution of certain pools and pathways in the soil more closely, for example by distinguishing between mineralization of applied organic matter vs. originally present SOM. There are many studies using ^{15}N to follow the mineralization of residues (Sørensen, 2001; Bol *et al.*, 2003; Kuzyakov *et al.*, 2009; Hilscher & Knicker, 2011) or fertilizers (Van *et al.*, 1985; Schulten & Schnitzer, 1997; Bengtsson *et al.*, 2003; Burger & Jackson, 2003) in soil. However, tracer studies comparing manures and digestates are scarce, and to our knowledge no soil incubations have been performed of a sequence of labeled manure bioenergy by-products.

The aim of the present study was to quantify GHG emissions and evaluate C and N dynamics of soils amended with cattle manure and digestate and biochar derived from that manure. A ^{15}N labeled cattle manure was prepared, from which we obtained an anaerobic digestate that was subsequently pyrolysed. In theory, these three products represent increasingly stabilized organic matter, decreasing the bioavailability of C and N. Specifically, we tested the hypotheses that, compared with manure, (1) the digestate and the biochar have increasingly higher C sequestration potential; (2) N mineralization of the digestate and biochar are progressively lower, so that these residues will be less effective as short-term fertilizers; and (3) emissions of N_2O from amended soils decline over the sequence.

Materials and methods

Soils and residues used for incubations

Two typical agricultural soils in the Netherlands with different properties were selected for the incubation experiments: a loess soil and a sandy soil. The loess soil (20% sand, 61% silt, and 19% clay, pH 6.4; C : N ratio 18) was collected at the arable farm 'Wijnandsrade' (50°54′N, 5°52′E). The sandy soil (75% sand, 23% silt, and 2% clay; pH 4.7; C : N ratio 11) was collected at the experimental farm 'Droevendaal' (51°59′N, 5°39′E). Both soils were sampled from the 0–25 cm layer of the arable field. Air-dried soils were sieved (<7 mm) and stored (20 °C) until the beginning of the experiments.

Residues of three successive stages of biomass processing were used (details in Table 1):

^{15}N labeled cattle manure (0.551 atom%^{15}N);

^{15}N labeled anaerobic digestate from the above manure (0.759 atom%^{15}N);

^{15}N labeled biochar from the above digestate (0.656 atom %^{15}N);

The ^{15}N labeled cattle manure was prepared by feeding ^{15}N labeled rye grass (*Lolium perenne* L.) to a nonlactating cow (Powell & Wu, 1999). The ^{15}N enrichment of the rye grass was 18.4 atom% excess. The excrements of the cow (urine and feces) were collected over a 7-day period, and the urine and feces mixed.

A 10 L subsample of the ^{15}N labeled manure was anaerobically digested at 35 °C in an 11 L continuously stirred tank reactor for 44 days at the Department of Environmental Technology, Wageningen University, the Netherlands.

A 5 L subsample of the digestate was air-dried at 25 °C for 2 days and consecutively pyrolysed (flash pyrolysis) at 500 °C (PyRos-process patented by TNO NL99/00688) in the Laboratory of Thermal Engineering, University of Twente, the Netherlands.

Manure and digestate were freeze-dried. All residues were ground and sieved (<0.5 mm) before application to avoid a particle size effect.

Table 1 Main chemical properties of residues used for incubation with soil

	Manure	Digestate	Biochar
TOC (% dry weight)	37.14	30.36	31.73
TN (% dry weight)	1.52	2.44	1.71
TOC : TN	24.4	12.5	18.6
Atom% ^{15}N	0.551	0.759	0.656

TOC, total organic carbon; TN, total nitrogen.

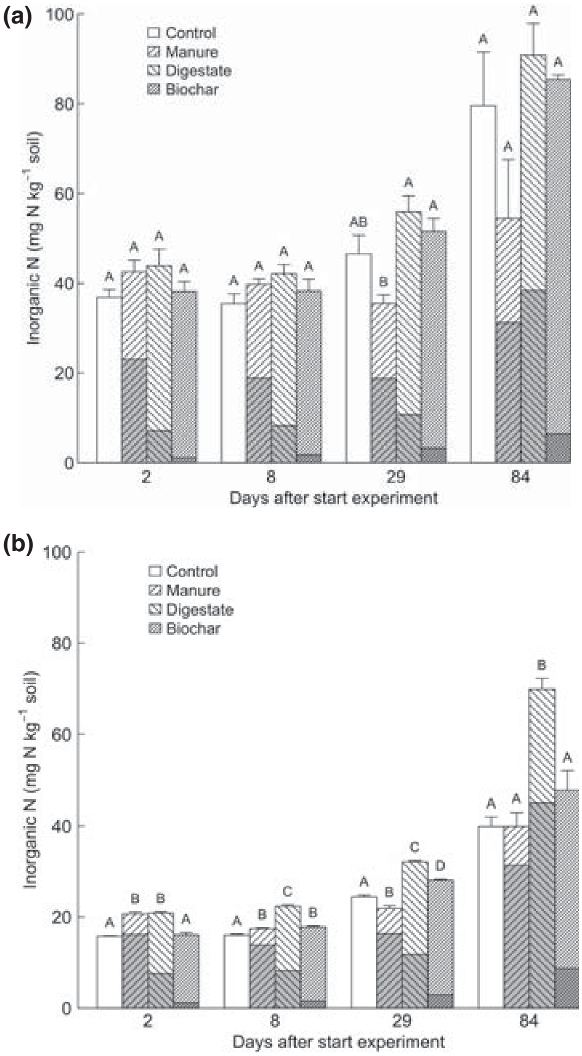

Fig. 4 Inorganic nitrogen (NH_4^+, NO_2^-, and NO_3^-) in loess soil (a) and sandy soil (b), 2, 8, 29, and 84 days after application of manure, digestate, and biochar. The gray segments indicate the amount of inorganic N derived from mineralization of the amended residue as determined by isotopic tracing. Letters show significant differences between treatments on each separate day according to the Tukey test ($P < 0.05$).

soil on day 2, compared with a maximum of 7% in loess (biochar).

The amount of N in microbial biomass, recorded in the end of the incubation, was highest for manure amended soils (Table S4). However, differences were not significant ($P > 0.05$).

Discussion

Stability of carbon

Anaerobic digestion and pyrolysis increasingly stabilized organic matter by reducing the easily biodegradable C fractions. As a result, the mineralization rate of remaining C was low, which confirms the findings of previous independent studies on digestates and biochars (Clemens *et al.*, 2006; Zimmerman, 2010). Although C in digestates of animal slurries is typically found to be more stable than C in untreated slurries (Marcato *et al.*, 2009), differences are not always seen (e.g. Bertora *et al.*, 2008). Cayuela *et al.* (2010) found anaerobic digestion of pig slurry to reduce C loss from 57% to 40% over 60 days, but recorded no difference between cattle manure and its digestate (38% and 36% of added C lost as CO_2, respectively). The observed variability in recalcitrance to mineralization of digestion residues may therefore be explained by dissimilar feedstock properties and (suboptimal) digestion conditions (Angelidaki & Ahring, 2000).

Previous incubation studies have shown high recalcitrance of biochar to degradation in soil (Spokas & Reicosky, 2009; Zimmerman, 2010). However, flash pyrolysis chars are known to display higher reactivity than those prepared by slow pyrolysis (Zhang *et al.*, 2009). Expressed as percentage of C added, biochar amendment resulted in biochar C losses of 14% in loess and 6% in sandy soil during our experiment (78 days), which agrees with the findings of Bruun *et al.* (2012) for flash pyrolysis biochar.

The present study did not discriminate between evolution of biochar C (BC) and native soil organic carbon

Table 3 Percentage of inorganic N (NO_3^-, NO_2^-, and NH_4^+) derived from the added by-product as deduced from the isotopic dilution (relative to the total mineral N extracted from soil).

	Incubation time (days)				
Treatment	2	8	29	84	ANOVA
Loess soil					
Manure	54.1 Aab	47.5 Aa	52.8 Aab	57.4 Ab	*
Digestate	16.2 Ba	19.5 Ba	19.2 Ba	42.2 Bb	***
Biochar	3.1 Ca	4.5 Cab	6.4 Cbc	7.4 Cc	**
ANOVA	***	***	***	***	
Sandy soil					
Manure	78.1 Aa	79.6 Aa	74.7 Aa	78.8 Aa	ns
Digestate	36.3 Ba	36.8 Ba	36.8 Ba	64.2 Bb	***
Biochar	7.2 Ca	8.8 Ca	10.3 Ca	18.3 Cb	***
ANOVA	***	***	***	***	

One-way analysis of variance (ANOVA) was applied to detect significant differences among treatments and time. Values in the same column followed by the same letter are not significantly different according to the Tukey test ($P < 0.05$).
ns, not significant.
$^{***}P < 0.001$;
$^{**}P < 0.01$;
$^{*}P < 0.05$.

(SOC). Studies that separate BC degradation from the effect of biochar on SOC degradation by isotopic labeling (Kuzyakov *et al.*, 2009), or by subtracting abiotic degradation of BC from measured C loss in soil (Spokas & Reicosky, 2009), show that degradation of BC itself is very low. The role of biochar as means for C sequestration, however, requires that SOC mineralization should not be enhanced instead. So far, the great diversity of biochars in a wide range of different circumstances has not conclusively settled this issue (e.g. Wardle *et al.*, 2008; Singh *et al.*, 2010), largely because of the lack of long-term data. Recently, an extensive incubation study by Zimmerman *et al.* (2011) led to the hypothesis that as biochars mature in soil, positive priming (i.e. enhanced SOC mineralization) declines and is dominated by negative priming (i.e. sorption of SOC onto biochar surfaces), leading to a net stabilization of biochar plus SOC.

The dissolved organic carbon (DOC) pool in soil is usually strongly and positively correlated with CO_2 respiration (Haynes, 2005). Despite this, typically only 10–40% of DOC is observed to be readily degradable, probably owing to soluble humic substances that are relatively recalcitrant (ibid.). In our experiment, biochar amendment increased EOC (a measure of DOC) to the same extent as the other treatments (Fig. 3, day 2). However, the modest decline in EOC of biochar amended soils over the incubation period hints at a relative recalcitrance of extractable BC to degradation.

Nitrogen mineralization and nitrous oxide emissions

In accord with our second hypothesis, the proportion of soil inorganic N that originated from the residue was highest for manure, followed by the digestate and the biochar. Despite these significant differences, only the digestate resulted in increases of total inorganic N compared with nonamended soils ($P < 0.05$ in sandy soil). For manure amended soils, net mineralization (residue + soil) was equal to that of the control, whereas high amounts (57% and 79%, loess/sandy soil) derived from the residue. Conversely, biochar application did not prevent mineralization of native N, and mineralization of N from biochar, although small, was purely additive to soil N mineralization.

The evolution of N after digestate amendment exhibited a combination of both patterns. The apparent 'suppression' of soil N mineralization by manure and digestate amendment suggests commutability of sources of N for microbial assimilation. Immobilization as a fate for nitrogen upon residue amendment should also be considered, as microbial biomass N tended to be elevated in the manure treatments. The absence of significance between microbial biomass of the different treatments may be the result of a release of inorganic N by cell lysis or microbial osmoregulation following the rewetting and thawing events, which is supported by the high increases of inorganic N between day 29 and 84 (Davidson, 1992; Unger *et al.*, 2010).

In our experiment, N evolution from the added residue was comparable between soils, whereas the contribution from the soil was consistently smaller in the sandy soil. The C : N ratio of the sandy soil is high compared with the loess soil (18 and 11, respectively). This could have increased the relevance of added residue N for total microbial N turnover (Booth *et al.*, 2005). In addition, the loess soil exhibited patterns of higher microbial activity overall, as measured mineralization rates were always higher (for both C as N).

The influence of biochar amendment on soil N_2O emissions is hitherto controversial. Several authors found that biochar soil application can significantly reduce N_2O emissions (Spokas & Reicosky, 2009; Cayuela *et al.*, 2010; Van Zwieten *et al.*, 2010; Zhang *et al.*, 2010). An increase in N_2O production after biochar amendment has been also reported (Clough *et al.*, 2010; Kammann *et al.*, 2011). The mechanisms involved are still rather speculative and could include many biotic and abiotic factors. In our study, the most probable mechanism behind N_2O production was denitrification in the loess soil. Probably the highest C availability in the manure led to higher denitrification rates than in the other treatments. Hence, emissions of N_2O were reduced over the bioenergy sequence, which is in agreement with our third hypothesis.

There are not many studies reporting the effects of flash pyrolysis biochar on N_2O emissions. In a recent study, Bruun *et al.* (2011) found that flash pyrolysis biochar from wheat straw increased N_2O emissions from a loamy soil at high moisture conditions. The higher reactivity of flash pyrolysis biochar in comparison with slow pyrolysis biochar could explain the increase of N_2O losses.

Nitrous oxide emissions from the sandy soil were negligible, which we relate to its higher aeration due to its texture and to its moisture level, which was kept lower (60% of WHC) than in the loess soil (70% of WHC). N_2O emissions are enhanced in moist soils, as long as nitrification is not inhibited by oxygen limitations due to reduced aeration (Linn & Doran, 1984). However, in anoxic conditions the denitrifying community is known to cause N_2O fluxes when nitrate availability is sufficient (Beare *et al.*, 2009). In line with this, N_2O emissions in loess declined during the drying phase, and subsequently peaked after rewetting to 80% WHC and after thawing the units.

Considerations for cattle manure management

Demographic and dietary developments globally cause an increase in animal production systems, generating a growing flow of secretion products that require disposal. During manure storage, transport and field application, odorous substances and GHGs are released to the environment, and cases of eutrophication are widespread (Oenema & Tamminga, 2005). Treatment of animal residues can considerably reduce the burden on the environment in these successive stages (Amon *et al.*, 2006; Clemens *et al.*, 2006; Bertora *et al.*, 2008; Möller & Stinner, 2009; Kaparaju & Rintala, 2011). Certain treatments may yield energy, such as biogas in the case of anaerobic digestion, which results in further GHG abatement through reduced demands for fossil fuel (Cantrell *et al.*, 2008; Holm-Nielsen *et al.*, 2009). In the use of agricultural residues for bioenergy production, only the chemical energy present in reduced carbon compounds is of interest; other compounds may be used to restore the fertility of soils. However, to balance depletion of SOM by agricultural practices, it is also desired to return a significant amount of C to the soil (Lal, 2005). In principle, this study showed that soil amendment with bioenergy by-products could compensate for the SOM inputs no longer provided when manure amendment is aborted. The stability of SOM in biochar and digestate treatments were high when considering their reduced C : N ratios, and an increased N supply was observed in the case of digestate.

Biochar is rapidly gaining recognition as a soil amendment, improving the fertility of soil through a range of short- and long-term processes (Antal & Grönli, 2003; Joseph *et al.*, 2010). Importantly, its recalcitrance is cause for the consideration of using biochar as a means for widespread carbon sequestration in soils. For most sources of organic matter, the scope for C sequestration is limited by the capacity of clay particles to stabilize SOM on the one hand (Six *et al.*, 2002), and the balance of C and N inputs and outputs on the other (Schlesinger, 2000; Khan *et al.*, 2007). Biochar has a much greater inherent stability (Masiello, 2004; Forbes *et al.*, 2006; Laird *et al.*, 2008), and modeled C balance studies have shown convincing net benefits (Gaunt & Lehmann, 2008; Woolf *et al.*, 2010). The present study raises, however, some questions on its use. In our study, the biochar C yield was approximately 33%. The total C of the cattle manure feedstock was first reduced by 50% through anaerobic digestion, which means that the amount of biochar C is 17% from the initial quantity, of which 84–93% is recalcitrant. Therefore, the C sequestration potential of cattle manure via the digestion-pyrolysis route, as judged by the 1-year humification coefficient obtained in this study, was only 15% compared with 53–55% for untreated cattle manure. This underpins the importance of long-term behavior of residues in soil when evaluating the benefits of competing C pathways.

The results found in this laboratory experiment cannot be upscaled to field conditions. Hence, our results should be considered as a relative rather than an absolute approach. However, our study clearly shows that bioenergy production from manure has important implications on the recalcitrance of C and N in the by-products. Through N tracing, we demonstrated that the release of N in soil derived from the by-product decreases with anaerobic digestion and even more after pyrolysis, which has important parallel effects, such as the reduction of N_2O emissions.

Acknowledgments

The authors are very grateful to Kealan Gell and Tania Fernandes for their valuable contribution in the anaerobic digestion experiment at the Department of Environmental Technology at WUR. Many thanks to Ali Imran and Gerrit Brem for the production of biochar at the University of Twente, to Gerard Ros for his assistance with the micro-diffusion procedure, to Gonzalo Gonzalez-Barberá for his valuable help with statistics and to Ron de Goede for supplying the labeled cattle manure. Maria Luz Cayuela was supported through a European Community Marie Curie Fellowship (Intra-European Fellowship for career development: FP7-PEOPLE-2007-2-1-IEF-Proposal No. 220868 – BEST) under which this study was performed. Thanks to two anonymous reviewers for their constructive comments.

References

Amon B, Kryvoruchko V, Amon T, Zechmeister-Boltenstern S (2006) Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems & Environment*, **112**, 153–162.

Angelidaki I, Ahring BK (2000) Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Science and Technology*, **41**, 189–194.

Antal MJ Jr, Grönli M (2003) The art, science, and technology of charcoal production. *Industrial & Engineering Chemistry Research*, **42**, 1619–1640.

Beare M, Gregorich E, St-Georges P (2009) Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. *Soil Biology and Biochemistry*, **41**, 611–621.

Bengtsson G, Bengtson P, Månsson KF (2003) Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry*, **35**, 143–154.

Bertora C, Alluvione F, Zavattaro L, Van Groenigen JW, Velthof G, Grignani C (2008) Pig slurry treatment modifies slurry composition, N_2O, and CO_2 emissions after soil incorporation. *Soil Biology and Biochemistry*, **40**, 1999–2006.

Bol R, Kandeler E, Amelung W, Glaser B, Marx M, Preedy N, Lorenz K (2003) Short-term effects of dairy slurry amendment on carbon sequestration and enzyme activities in a temperate grassland. *Soil Biology and Biochemistry*, **35**, 1411–1421.

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological Monographs*, **75**, 139–157.

Bousfield S, Hobson PN, Summers R (1979) A note on anaerobic digestion of cattle and poultry wastes. *Agricultural Wastes*, **1**, 161–164.

Bradbury NJ, Whitmore AP, Hart PBS, Jenkinson DS (1993) Modelling the fate of nitrogen in crop and soil in the years following application of ^{15}N-labeled fertilizer to winter wheat. *Journal of Agricultural Science*, **121**, 363–379.

Brookes P, Landman A, Pruden G, Jenkinson D (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Bruun EW, Müller-Stöver D, Ambus P, Hauggaard-Nielsen H (2011) Application of biochar to soil and N_2O emissions: potential effects of blending fast pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science*, **62**, 581–589.