OA Microbiology
Open Access

Review

Microbial Ecology

Tracking N-cycling genes in biochar-supplemented ecosystems: A perspective

CH Orr[1], TK Ralebitso-Senior[1*]

Abstract

Introduction

Since biochar has the potential to mitigate climate change and enhance agricultural outputs, new research is exploring its dual role relative to greenhouse gas emissions from agronomic soils, with particular focus on nitrous oxide (N2O). It is well accepted that definitive investigations of sustainable contemporary biochar applications in different (bio) technologies must be underpinned by combined physico-chemical and microecophysiological analyses. Nevertheless, recent nitrogen cycle research has measured principally the occurrence and emission of different N species to then infer shifts in microbial activity in response to biochar augmentation, with a few emerging studies assessing its effects on the functional genes/communities. As a result, a wide scope for critical and exciting research exists. This must be informed by comprehensive multidisciplinary studies of the dynamics of functional N-cycle genes, enzymes, strains and communities across different ecosystems and environmental biotechnologies – agriculture, contaminant remediation, wastewater treatment, malodorant gas biofiltration and landfill. This review aims to summarize the state-of-the art and highlight critical research that is required to assess the effect of biochar addition on N-cycling in different ecosystems.

Conclusion

We conclude that despite emerging research there are still critical knowledge gaps on the microbial response to biochar, which need to be addressed before the material can be applied in specific key environmental biotechnologies.

*Corresponding author
Email: K.Ralebitso-Senior@tees.ac.uk

[1] Teesside University, Middlesbrough, United Kingdom

Introduction

Char/biochar has multiple and memorable descriptors including: (i) charcoal used to sequester carbon and supplement soil; (ii) a recalcitrant carbon-rich compound that is a by-product of pyrolysis of organic wastes and other biomass; (iii) "black is the new green"; and (iv) the new "black gold" (e.g.[1,2]).

These encapsulate its extensive, but as yet little, exploited potential to address several key challenges across the spectrum of climate change mitigation, global agricultural productivity demand, sustainable carbon-neutral energy production, and contaminant attenuation.

Although the combined and enhanced roles of biochar and soil microbial populations are recognised, a considerable paucity of knowledge remains on its impacts on microbial diversity and functional response, in general, and nitrogen cycling, in particular.

Depending on the feedstock and pyrolysis production conditions, different biochars may be applied to pristine (agricultural) land or used for different waste managements[2] (Figure 1). Therefore, this perspective considers the current understanding and identifies the knowledge gaps in biochar-induced microbial dynamics of the N-cycle in agronomic and non-agronomic contexts. The latter exploitation potentials include environmental biotechnologies such as contaminated soil/sediment remediation, wastewater treatment, malodorant gas biofiltration and landfill gas and leachate attenuation.

The aim is to present a cohesive and succinct synopsis of key findings published up to 2014 and extend existing comprehensive reviews related directly[3,4,5,6,7] and indirectly[8,9,10] to nitrogen-biochar interactions.

Generally, focussed overlaps of current discussions identify viable, innovative and empirical research, and justify new studies, that must underpin informed and sustainable exploitation of biochar relative to the nitrogen biogeochemical cycle.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Agronomic contexts

Quilliam et al.[11] reviewed critically biochar beneficiation of agricultural soils through a range of mechanisms. Protracted C storage, reduced nutrient leaching, pesticide adsorption and soil physico-chemical parameter effects, which may, subsequently, have positive impacts on microbial activity, were all identified. Collectively, these may lead to increased crop yields, which are often attributed to increased activity and diversity of specific functional soil microorganisms[12].

After water, nitrogen is a key plant growth limiter that is needed in relatively high concentrations (20-40 Kg ha^{1} every 3 months) by most agricultural crops[13]. For nitrogen to be used by crops it must be available in the correct moiety.

Consequently, considerable energy inputs are necessitated by the Haber Process to manufacture ammonia-based fertilizers and so incur a high carbon footprint. Therefore, biochar-promoted fixation to increase soil nitrogen capital (Figure 1) could reduce this dependency provided that the potential attendant benefits of inimical N2O emission mitigation are proven.

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Orr CH, Ralebitso-Senior TK. Tracking N-cycling genes in biochar-supplemented ecosystems: A perspective. OA Microbiology 2014 Jan 18;2(1):1.*

OA Microbiology
Open Access

Review

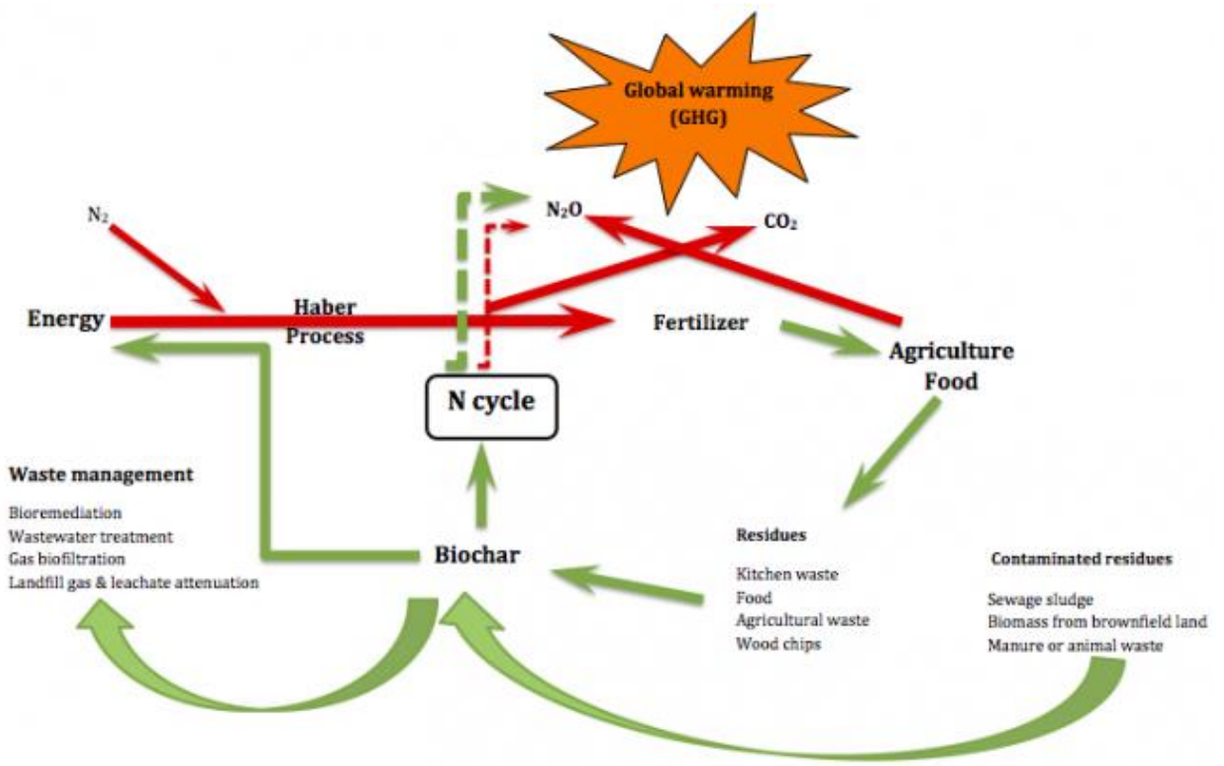

Figure 1: Climate change imperatives: Potential nitrogen cycle-biochar interactions. (Red arrows indicate inimical impacts, Green arrows highlight positive exploitation potential, Dashed arrows identify potential to mitigate (green) or increase (red) N2O emission.).

Previous publications[3,4,5,7] have reported nitrogen species amount and type differences resulting from biochar applications with nitrate and ammonia absorption, decreased nutrient leaching and reduced emissions of greenhouse gas nitrous oxide.

The nitrogen cycle is characterised by three principal microbial-mediated processes: nitrogen fixation; nitrification; and denitrification.

Consequently, several genes that encode key enzymes are identified routinely to measure changes in their respective functional microbial communities. The most common are: nifH encoding nitrogenase, essential for nitrogen fixation; amoA encoding ammonium monoxygenase, for nitrification; and nirS, nirK and nosZ, for different stages of denitrification. The nir genes encode nitrate reductase, for reducing nitrate-N to nitric oxide, while nosZ encodes nitrous oxide reductase, which reduces N2O to N2. Most published investigations of the effects of biochar on N cycling have, however, quantified different N species and, through data extrapolation, assumed microbial activity changes[5,14,15]. For direct microbial response studies, terminal-restriction fragment polymorphisms (TRFLP), 454 sequencing and real-time or quantitative polymerase chain reaction (qPCR) were the most common approaches but these recorded variable results (Table 1).

Wang et al.[16] researched biochar supplemented pig manure compost piles and correlated nosZ copy number increases to short-term N2O emission decreases. Harter et al.[17], Ducey et al.[18] and Anderson et al.[19] used qPCR to measure nitrogen cycling gene copy numbers in various experiments. Specifically, Anderson et al.[19] determined nirS, nirK and nosZ genes in a field experiment and recorded increased nirS and nosZ copies when biochar was added with exogenous nitrogen. Copy number increases for nirS occurred within the first 10 days whereas those for nosZ were recorded > 20 days. Ducey et al.[18] and Harter et al.[17] measured nifH, amoA, nirK, nirS and nosZ in pot experiments with the former team reporting increases in the nirS and nifH copy numbers and the latter observing an increase in nosZ.

Generally, these studies reported consistent increases in nitrogen cycling gene copy numbers in response to biochar augmentation in the presence of inorganic N. This mirrors conventional farming practices but suggests that biochar addition could have a lesser impact in low-input and organically managed farmland.

Although qPCR analysis results are indicative of functional microbial community activity, they do not show diversity changes hence TRFLP and 454 sequencing approaches have been applied[19,20,21]. Dempster et al.[21], for example, used community level physiological profiling to assess the heterotrophic microbial community, in general, and TRFLP for amoA gene-based nitrification, specifically.

Biochar supplementation effected structure changes and decreased

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Microbiology
Open Access

Review

Table 1: Summary of biochar type, application regime, analyses, N-cycle genes targeted and N-molecule dynamics.

Biochar substrate	**Pyrolysis conditios -T and residence time**	**Biochar application regime**	**Soil type or properties**	**Analyses**	**N-cycle genes**	**N-molecule dynamics**	**References**
Monterey pine (*Pinus radiate*)	500°C	0, 15 and 30 t ha^{-1}; Field plots before soil was used for pot experiments	Templeton silt-loam	TRFLP & 454 sequencing of 16S rRNA	n/a	n/a	Anderson et al 2011 **[20]**
Coppiced woodlands	500°C	0, 3 and 6 kg m^{-1}; 3 and 14 months	Silty-loam growing wheat (cv. *Neolatino*)	Measured emissions of N_2O, CH_4 and CO_2	n/a	Lowered N_2O emissions but did not affect net nitrification rates	Castaldi et al 2011 **[27]**
Monterey pine (*Pinus radiate*)	500°C	Lysimeters; Various ratios of biochar and biosolids	Templeton silt-loam; Ashley Dene silt-loam	Chemical analysis	n/a	Reduction in nitrate leaching	Knowles et al 2011 **[28]**
Jarrah wood (*Eucalyptus marginata*)	600°C for 24 hours	0, 5, 25 t ha^{-1}; With organic (500 kg ha^{-1}) or inorganic (100 kg ha^{-1}) N; Wheat field	Grey Orthic Tenosol	TRFLP of *amoA* and CLPP	*amoA*	Decrease in N mineralization, change in AOB community structure when biochar added in combination with inorganic N.	Dempster et al 2012 **[21]**
Green waste	550°C	0.5%(w/w) anaerobic digestate, rapeseed meal, bioethanol residue or biochar; With organic supplements; Pot trials; 30 days	Arable Typic Dystrudept	Measured C, N, nitrate, ammonium, P, microbial biomass C and enzyme activity	n/a	No effect	Galvez et al 2012 **[29]**
Silage maize	350°C and 550°C	10 g fresh biochar kg^{-1} dry soil; Pot experiments; 168 hours after ^{15}N addition	Arable loamy sand soil	^{15}N tracing	n/a	Increased N mineralization, nitrification and ammonium consumption	Nelissen et al 2012 **[14]**
Rice husks	450°C	0, 10, 25 and 50 t ha^{-1}; Pot experiments; With and without nitrogen fertilizer	Upland Orthic Anthrosols; Paddy Stagnic Anthrosols	Emissions of CO_2, CH_4 and N_2O	n/a	Reduction in N_2O emissions when applied with N fertilizer	Wang et al 2012 **[15]**
Wheat straw	350-550°C	0, 10, 20 and 40 t ha-1	Hydroagric Stagnic Anthrosol	Emissions of CO2, CH4 and N2O	n/a	Decreased N2O emissions	Zhang et al 2012 [26]
Brush	500°C	2% (w/w)	15 different agricultural soils	15N tracing	n/a	Decreased N2O emission	Cayuela et al 2013b APR [5]
Switchgrass (Panicum virgatum)	350°C and steam activated at 800°C	1%, 2%, 10% (w/w); Pot trials; 6 months	Portneuf (coarse-silty, mixed, superactive, mesic Durinodic Haplocalcid)	qPCR	nifH, amoA, nirS, nirK, nosZ	Increases in nifH and nirS	Ducey et al 2013 [18]

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Orr CH, Ralebitso-Senior TK. Tracking N-cycling genes in biochar-supplemented ecosystems: A perspective. OA Microbiology 2014 Jan 18;2(1):1.*

OA Microbiology
Open Access

Review

Table 1: (continued)

Green waste	700°C	0%, 2%, 10% (w/w); 202 g field wet soil; Laboratory-scale microcosms	Calcaric Leptosol with ~50% (w/w/) gravel	qPCR;	nifH; amoA; nirK; nirS; nosZ	N2O emission reduced	Harter et al 2013 [17]
Chipped trunks of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L.	450°C for 48 hours	0, 25 and 50 t ha^{-1}; Field plots; 3 years	Eutric Cambisol (sandy clay loam)	Quantify nodules on plant roots; Nitrogen fixation using acetylene reduction assay	n/a	Short term increase in nitrogen fixing ability which diminished over time	Quilliam et al 2013 MAY **[30]**
Anaerobically digested pig manure; Sitka Spruce (*Picea sitchensis*)	600°C	0 and 18 t ha^{-1}; With pig manure	Acid Brown Earth	Measured N_2O and CO_2 emissions	n/a	Increased N_2O emissions	Troy et al 2013 **[31]**
Bamboo	600°C	0 and 60 kg biochar + 1200 kg pig manure and 800 kg bulking agent; 82 days	n/a	qPCR and N_2O emission measurement	*nosZ, nirK, nirS*	Increased nitrite concentrations and N_2O emissions	Wang et al 2013 **[16]**
Pinus radiata	450°C	0, 15 and 30 t ha^{-1}; In presence and absence of bovine urine; Field plots; 70 days	Templeton silt-loam	TRFLP & 454 sequencing (16S rRNA), & qPCR (N cycling)	*nirS, nirK, nosZ*	Increases in nitrifiers and denitrifiers number	Anderson et al 2014 **[19]**
Grass	400°C	0, 10, 50 and 120 t ha^{-1}; Pot experiments; With red clover (*T. pratense* L.)	Grassland soil	^{15}N tracing and nodule count	n/a	Increased N fixation	Mia et al 2014 **[32]**
Willow; Pine; Maize; Wood mixture	350-650°C	20 t ha^{-1}; With and without different fertilizers; Pot experiments; 14 days	Silt-loam or Luvisol	Measured emissions of N_2O and NO	n/a	Increased N_2O and NO emissions when applied in combination with urea and nitrate fertilizers	Nelissen et al 2014 **[33]**

diversity in the total heterotrophic community with the nitrifying associations affected only when organic/inorganic N sources were added. Anderson et al.[19,20] applied TRFLP and 454 sequencing of the 16S rRNA gene to monitor shifts in microbial diversity. Although both studies showed no overall microbial community structure changes, the earlier investigation[20] recorded relative abundance differences of specific bacterial families in response to biochar, with some of the genera and/or species involved in nitrogen cycling. Thus, the biochar increased the denitrification families/glades of Bradyrhizobiceae and Hyphomicrobiaceae. Also, abundance decreases and increases were recorded for nitrifying and nitrogen-fixing communities, respectively.

Nonetheless, the wide distribution of nitrogen fixing and denitrifying genes across and within most microbial phyla preclude assumptions that N cycling species are present when the function has been recorded and identified at family level. For example, Anderson et al.[19] identified operational taxonomic units at genus level and recorded increases in Phyllobacterium, Bradyrhizobium and Hyphomicrobium following biochar and exogenous N additions.

These genera have been associated with nitrogen fixation and denitrification but identification at the species level would be essential for detailed and conclusive analyses. Therefore, a range of relevant microecophysiology methods (see for example Ennis et al.[8]) should target N cycling genes, not the 16S rRNA gene, to determine diversity, structure and

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Orr CH, Ralebitso-Senior TK. Tracking N-cycling genes in biochar-supplemented ecosystems: A perspective. OA Microbiology 2014 Jan 18;2(1):1.*

OA Microbiology
Open Access

Review

composition changes of both the bacterial and archaeal species underpinning nitrogen dynamics following biochar augmentation.

Before 2014, studies that measured community composition and structure changes mainly used short-term pot experiments, which may be unrepresentative of field scale responses. In particular, biochar addition to soil often results initially in a transient pH increase and leads potentially to brief and unsustained community shifts[16].

Therefore, pilot-scale and long-term investigations, underpinned by relevant complementary physico-chemical and microecophysiology analyses, including ^{15}N tracer studies, are critical to establish the diversity and functional responses of the microbial community N-cycle drivers in biochar-augmented ecosystems.

The rhizosphere

Understanding rhizospere microbial ecology in response to biochar application is essential for agriculture and all phyto-based environmental biotechnologies. Studies must explore N availability following biochar addition[4] and, hence, impacts on rhizospheric interactions, plant biomass and agricultural yield potential, and contaminant attenuation. Findings from multiple seminal studies and reviews (e.g. de Bruijn[22]) have proposed/ illustrated elegantly the use of cutting-edge microecophysiology and spectroscopy tools to explore rhizosphere microbial community dynamics and nutrient uptake. Consequently, plants such as clover (Trifolium spp), Arabidopsis sp., wheat (Triticum sp.) and soybean (Glycine max (L.) Merr.) have been used to explore how rhizospheric manipulations enhance plant yield/ biomass and how the rhizosphere microbiome responds to different plant species and/or their stages of growth. Some of these investigations have then explored the role of the nitrogen cycle in particular.

For example, Faragová et al.[23] researched ammonifying, denitrifying and nitrifying bacteria in wild-type and transgenic alfalfa (Medicago sativa) by colony forming unit analysis on selective media. Such studies can, therefore, serve as templates for focussed research on N-cycle dynamics in the presence of biochar and be extended to include other functional groups, i.e. archaea.

Although not in a N-cycle context, Quilliam et al.[11] used ^{13}C-glucose and scanning electron microscopy to probe biochar as a habitat provider for soil microbial communities. They added to the recognition that biochar supplementation changed the physico -chemical properties, labile substrates and, subsequently, soil-plant-microbe interactions of the surrounding soil, which they termed the "charosphere".

Thus charosphere-specific studies should be expanded to the use of natural fertilizers, especially for strategies that entail manipulations and/or enhancements of rhizosphere functional microbial activities. This approach would complement on-going studies of nifH gene abundance and diversity relative to site-/soil-specific soil chemistry[24] and N2O flux in response to biochar application to chemical fertilizer augmented soil[6].

Non-agronomic contexts

According to Clough and Condron[3], future research should aim to understand "… N transformation in soils, both chemical and biological mechanisms, and the fate of N applied to biochar treated soils." This was also proposed by other researchers[17] specifically to investigate and mitigate the potential release of GHGs, including methane and nitrous oxide.

Green House Gas emission is not unique to soil applications since it is an issue in other biotechnologies where biochar will, inevitably, be exploited. It is reasonable to propose, therefore, that biochar augmentation studies are extended to N-based molecule cycling dynamics in: i) contaminated ecosystems (soil, sediment, aquatic); ii) wastewater treatment; iii) malodorant gas biofiltration; and iv) landfills.

Contaminated sites

As reported in previous comprehensive reviews (e.g. Lehmann and Joseph[1], Clough and Condron3), biochar can enhance aeration and soil porosity, which are key variables for N2O production and diffusion. Thus, studies on catabolic genes, enzymes, strains and communities for N-based contaminants such as herbicides (e.g. atrazine), fungicides (e.g. creosote) and nitrates from different anthropogenic activities/products (e.g. fertilizers, animal waste, manure, sewage) should characterise nitrogen cycling dynamics of biochar-supplemented remediation programmes. These should entail culture-based and omic techniques including relevant tracer ^{15}N labelling protocols as proposed, for example, by Ennis et al.[8] and Butterbach-Bahl et al.[10].

Also, phytoremediation rhizospheric interactions, particularly for plant species that are known or unknown nitrogen fixation facilitators, would mandate particular scrutiny.

Wastewater treatment and waste gas biofiltration

For decades, biotrickling filters have treated successfully both liquid and gaseous wastes. They can, therefore, be used to investigate biochar potential as an additional biofilm support[9]. For example, bamboo-derived biochar effected chemical adsorption of aqueous NH3 while palm-oil char (500°C) recorded a 0.70 mg g^{1} adsorption capacity efficiency when exposed to 6 µL L^{1} gaseous NH3 (see Clough and Condron[3]). These studies could be extrapolated to identify potential physico-chemical removal of ammonia-N in wastewater and malodorant gasses. Thus, ecogenomic analyses should be added for repeated or similar experimental protocols to characterise the N-cycle mechanisms from a microbial community functional, compositional and structural perspective.

Comprehensive analyses must also consider the presence of residual biochar products that may be -static or –cidal to specific functional genes/ enzymes/strains underpinning the N-cycle.

Since biochars are generally alkaline, they can be exploited, potentially, as support materials with intrinsic

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Microbiology
Open Access

Review

buffering capacities[6] for low pH waste gas biofiltration. As for soils, role investigation of key parameters (pH and H2S) for microbial nitrous oxide production would be essential for biochar-based malodorant gas biofiltration.

Specifically, these variables change depending on the source industry, molecules, concentrations and physico-chemical properties of the specific waste gas[9].

Landfills

According to Harter et al.[17], 60% (v/v) of anthropogenic N2O, which accounts for 8% (v/v) of global GHG emissions, originates from agricultural activities. Various studies have reported different landfill emissions that range from 0.0017 to 428 mg N2O-N m^{-2} h^{-1} (or 20–200 g CO2 eq. m^{-2} h^{-1}) depending on several parameters including landfill category, age, type of cover and location (e.g. Harboth et al.[25]). Although landfill contributions to the global N2O capital are considerably lower than from agronomic activities, its global warming potential is reported to be 289 kg CO2-e compared to 1 and 72 kg CO2-e for CO2 and CH4, respectively.

Therefore, findings that show reduced nitrous oxide (and methane) emissions in response to biochar application[1,15, 17,26] justify exploitation of the material for the attenuation of landfill leachate and gas. These applications must, however, be preceded by focussed and concerted research of the effects of biochar on the biogeochemical cycling of N2O and all N-based molecules specifically in landfills. Also, such studies should be considered within the context of whether landfill cover soil that is also inoculated with earthworms would exacerbate or mitigate nitrous oxide emission[6].

Conclusion

Emerging studies and discourse on the dynamics of microbial communities in response to biochar reflect the recognition of critical knowledge gaps in general but specifically for the nitrogen biogeochemical cycle. These have, subsequently, identified a wide, novel and interesting scope of research with potential for cutting-edge studies of the functional microorganisms at the genetic level. Addressing this paucity should underpin sustainable and informed contemporary applications of biochar to ensure that the benefits outweigh the disadvantages, e.g. greenhouse gas emissions, including nitrous oxide, are reduced and not increased. Independent of the ecosystem and/or site conditions, shifts in the functional microbial genes/enzymes/communities and, hence, the mechanisms of N-cycling, depend on the application regime and physico-chemical properties of each char. It is well established that these are, in turn, dictated by feedstock, pyrolysis conditions and ageing.

Therefore, while some of the knowledge established thus far for agronomic soils may be transferable, key unique paucity will mandate investigations that are specific to individual environmental biotechnologies.

References

1. Lehmann J, Joseph S, editors. Biochar for environmental management: science and technology. Earthscan. 2009.
2. Sohi S, Lopez-Capel E, Krull E, Bol R. Biochar, climate change and soil: a review to guide future research. CSIRO Land and Water Science Report 05/09. CSIRO. 2009 Feb.
3. Clough TJ, Condron LM. Biochar and the nitrogen cycle: introduction. J Environ Qual. 2010 May;39:1218-23.
4. Spokas KA, Novak JM, Venterea RT. Biochar's role as an alternative N-fertilizer: Ammonia capture. Plant Soil. 2012 Jan:350:35-42.
5. Cayuela ML, Sanchez-Monedero MA, Roig A, Hanley, K, Enders A, Lehmann J. Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions. Scientific Reports. 2013 Apr;3:1732-39.
6. Cayuela ML, van Zwieten L, Singh, BP, Jeffery S. Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. Agric Ecosyst Environ. 2013 Oct;191:5-16.
7. Clough TJ, Condron LM, Kammann C, Müller C. A Review of biochar and soil nitrogen dynamics. Agron J. 2013 Apr;3:275-93.
8. Ennis CJ, Evans AG, Islam M, Ralebitso-Senior TK, Senior E. Biochar: carbon sequestration, land remediation and impacts on soil microbiology. Crit Rev Environ Sci Technol. 2012 Oct;42(22):2311-64.
9. Ralebitso-Senior TK, Senior E, Di Felice R, Jarvis K. Waste gas biofiltration: advances and limitations of current approaches in microbiology. Environ Sci Technol. 2012 Aug;46:8542-73.
10. Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Phil Trans R Soc B. 2013 May;368(1621): 20130122.
11. Quilliam RS, Glanville HC, Wade SC, Jones DL. Life in the 'charosphere' – Does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem. 2013 Jun;65:287-93.
12. Farrell M, Kuhn TK, Macdonald LM, Maddern TM, Murphy DV, Hall PA, Pal Singh B, Baumann K, Krull ES, Baldcock JA. Microbial utilisation of biochar-derived carbon. Sci Total Environ. 2013 Nov;465:288-97.
13. Vitousek PM, Aber J, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman GD. Human alterations of the global nitrogen cycle: causes and consequences. Ecol Appl. 2000 Aug;7:737.
14. Nelissen V, Rütting T, Huygens D, Staelens J, Ruysschaert G, Boeckx P. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biol Biochem. 2012 May; 55:20-7.
15. Wang J, Pan X, Liu Y, Zhang X, Xiong Z. Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. Plant Soil. 2012 Nov; 360(1-2):287-98.
16. Wang C, Lu H, Dong D, Deng H, Strong PJ, Wang H, Wu W. Insight into the effects of biochar on manure

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Microbiology
Open Access

Review

composting: Evidence supporting the relationship between N2O emission and denitrifying community. Environ Sci Technol. 2013 Jun;47:7341-9.
17. Harter J, Krause H-M, Schuettler S, Ruser R, Fromme M, Scholten T, Kappler A, Behrens S. Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J. 2013 Mar;8:660-74.
18. Ducey TF, Ippolito JA, Cantrell KB, Novak JM, Lentz RD. Addition of activated switchgrass biochar to an aridic subsoil increases microbial nitrogen cycling genes abundances. Appl Soil Ecol. 2013 Jan;65:65-72.
19. Anderson CR, Hamouts K, Clough TJ, Condron LM. Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does not alter relative proportions of nitrogen cycling bacteria. Agric Ecosyst Environ. 2014 Jun;191:63-72.
20. Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia. 2011 Jul;54:309-20.
21. Dempster DN, Gleeson DB, Solaiman ZM, Jones DL, Murphy DV. Decreased soil microbial biomass and nitrogen mineralization with Eucalyptus biochar addition to a coarse textured soil. Plant Soil. 2012;354;311-24.
22. de Bruijn FJ, editor. Molecular microbial ecology of the rhizosphere: Volume 1 & 2. John Wiley & Sons; 2013.
23. Faragová N, Faragó J, Drábeková J. Evaluation of abundance of aerobic bacteria in the rhizosphere of transgenic and non-transgenic alfalfa lines. Folia Microbiol. 2005 Aug;50 (6):509–14.
24. Collavino MM, Tripp HJ, Frank IE, Vidoz ML, Calderoli PA, Donato M, Zehr J, Aguilar OM. nifH pyrosequencing reveals the potential for location-specific soil chemistry to influence N2-fixing community dynamics. Environ Microb. 2014 Mar.
25. Harboth P, Fuβ R, Münnich K, Flessa H, Fricke K. Spatial variability of nitrous oxide and methane emissions from an MBT landfill in operation: strong N2O hotspots at the working face. Waste Management. 2013 Oct;33(10):2099-2107.
26. Zhang A, Bian R, Pan G, Cui L, Hussain Q, Li L, Zheng J, Zheng J, Zhang X, Han X, Yu X. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crops Res. 2012 Feb;127(27):153–60.
27. Castaldi S, Riondino M, Baronti S, Esposito FR, Marzaiolo R, Rutigliano FA, Vaccari FP. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. Chemosphere. 2011 Sep;85:1464-71.
28. Knowles OA, Robinson BH, Contangelo A, Clucas L. Biochar for the mitigation of nitrate leaching from soil amended with biosolids. Sci Total Environ. 2011 May;409:3206-10.
29. Galvez A, Sinicco T, Cayuela ML, Mingorance MD, Fornasier F, Mondini C. Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. Agric Ecosyst Environ. 2012 Oct;160:3-14.
30. Quilliam RS, DeLuca TH, Jones DL. Biochar application reduces nodulation but increases nitrogenase activity in clover. Plant Soil. 2013 May;366:83-92.
31. Troy SM, Lawlor PG, O'Flynn CJ, Healy MG. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol Biochem. 2013 Jan;60:173-81.
32. Mia S, van Groenigen JW, van de Voorde, TFJ, Oram NJ, Bexemer TM, Mommer L, Jeffery S. Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability. Agric Ecosyst Environ. 2014 Mar; 191:83-91.
33. Nelissen V, Saha BP, Ruysschaert G, Boeckx P. Effect of different biochar and fertilizer types on N2O and NO emissions. Soil Biol Biochem. 2014 Mar;70:244-55.

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Orr CH, Ralebitso-Senior TK. Tracking N-cycling genes in biochar-supplemented ecosystems: A perspective. OA Microbiology 2014 Jan 18;2(1):1.*