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Letter

# Methane-Derived Zero-Valent Carbon Solids Differentially Impact Model Nitrogen Cycling Bacteria and Significantly Inhibit Nitrification

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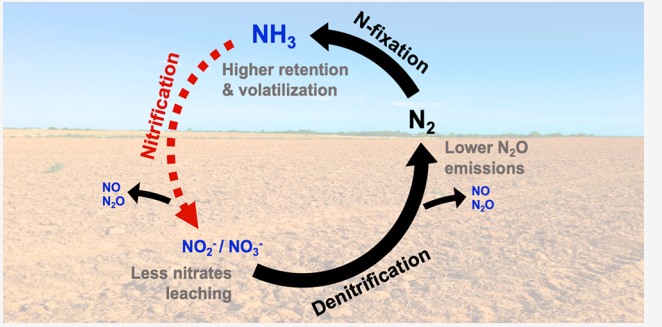
**ABSTRACT:** Pyrolysis of hydrocarbons holds promise for reducing $CO_2$ emissions associated with hydrogen production. This process co-produces solid zero-valent carbon (ZVC), which could be used similarly to biochar as an agricultural soil amendment. Since soil is the largest potential repository of ZVC, it is important to assess its potential impact on soil microbial ecosystem services, including the nitrogen cycle. Thus, we assessed how ZVC affects the growth and nitrogen cycling gene expression of three model bacteria: The nitrogen fixer was *Azotobacter vinelandii*, the nitrifier *Nitrosomonas europaea*, and the denitrifier *Pseudomonas stutzeri*. All bacteria attached to ZVC and charcoal (control), and neither noticeably affected the growth or activity of *A. vinelandii* and *P. stutzeri*. In contrast, ZVC significantly hindered the growth of *N. europaea*, down-regulated genes involved in ammonia oxidation, and reduced ammonium consumption. If such effects were pervasive in other nitroegn cycling soil bacteria, ZVC would potentially create a nitrogen cycle bottleneck by inhibiting nitrification, which would increase ammonia accumulation, possibly decreasing nitrogen fertilizer requirements but increasing $NH_3$ volatilization. This bottleneck would also restrict downstream processes like nitrate production, subsequent nitrate leaching, and denitrification, thus decreasing $NO_x$ emissions and emissions of the greenhouse gas $N_2O$. Overall, ZVC could impact nitrogen cycling, with important implications for environmental pollution and climate change.




**KEYWORDS:** *biochar, zero-valent carbon, nitrogen cycling, nitrification inhibition, $N_2O$ emissions mitigation*



## INTRODUCTION

Climate change due to fossil fuel combustion is motivating a transition toward a sustainable $H_2$ economy.[1] However, hydrogen in nature is rarely freely available,[2] and hydrogen production from water electrolysis may not be feasible in regions experiencing water scarcity. To bridge the gap between fossil fuels and hydrogen energy, the pyrolysis of hydrocarbons, including methane, has the potential to produce $H_2$ for energy without the $CO_2$ emissions associated with hydrocarbon combustion.[1] The only byproduct of methane pyrolysis is a potentially marketable zero-valent carbon (ZVC) solid that could improve the economics of industrial methane pyrolysis.[2,3] ZVC is being coined here as a convenient way of naming this carbon solid based on the theoretical stoichiometry of the pyrolytic conversion of methane to hydrogen and carbon ($CH_4 \rightarrow C_0 + 2H_2$). This does not imply that ZVC is a new allotropic form of carbon.

Among the different prospective applications for ZVC, the use of ZVC for soil amelioration could offer the scale required to match the current and future hydrogen markets. Additionally, other similar carbonized materials (e.g., charcoal and biochar) have already proven to provide benefits as agricultural soil amendments.[4,5] Although carbonized materials vary greatly, most have a relatively large surface area, highly aromatic structure, and neutral to alkaline pH.[6,7] These properties allow them to interact with abiotic and biotic soil factors in ways that often lead to improved soil fertility, soil structure, carbon storage capacity, and enhanced water retention.[5,8,9] However, despite its resemblance to other carbonized materials, further investigation is needed to understand potential unintended consequences and de-risk ZVC as a soil amendment.

Studies of the impacts of carbon solids often focus on improvements in soil properties, but soil microbes play a vital role in enhancing soil quality. Microbes are critical to maintaining soil structure, cycling nutrients, and moderating climate.[10] Microbial communities are also sensitive to changes in

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environmental conditions;[11,12] thus, adding carbonized materials could impact their ecosystem services. Specifically, biochar additions were reported to affect microbial nitrogen cycling processes, including nitrification and denitrification,[13,14] which are responsible for soil nitrogen losses often in the form of nitrogen oxides ($NO_x$) that contribute to smog formation and nitrous oxide ($N_2O$), a potent greenhouse gas. In several cases, biochar additions reduced nitrogen emissions from soils, with the proposed mechanisms including N immobilization, modified microbial gene expression and enzymatic activity, or toxic effects on nitrifier and denitrifier communities.[15] Hence, the suitability of ZVC as a soil amendment will depend in part on its impact on the microbial community and its associated ecosystem services, especially nitrogen cycling.

To address this critical knowledge gap, we examined how ZVC affects the growth and gene expression of model nitrogen cycling bacteria, focusing on nitrogen fixation (*Azotobacter vinelandii*), nitrification (*Nitrosomonas europaea*), and denitrification (*Pseudomonas stutzeri*). These archetypes are commonly used in laboratory studies[16,17] and play important roles in soil nitrogen transformations.[18−20] We performed experiments with washed and unwashed carbon solids to assess the impact of this potential post-production modification that has been reported to remove toxic contaminates[21] and improve functional properties.[22,23] Only *N. europaea* was highly susceptible to ZVC, which was more inhibitory than charcoal (control). These findings provide insight into a potential pathway by which ZVC could significantly hinder nitrification and impact the microbially driven nitrogen cycle and associated soil nitrogenous emissions.

## MATERIALS AND METHODS

**Preparation of Carbon Solids.** To produce ZVC, methane was thermally decomposed under anoxic conditions in a tubular reactor operating at temperatures between 1200 and 1500 °C and ambient pressure. The decomposition reaction occurred without a catalyst and was driven by the thermal energy transferred to the reactor through the reactor walls. Methane was injected together with a nitrogen carrier gas, with total flow rates selected to achieve a residence time between 0.5 and 10 s. While various types of ZVC could be produced, this particular ZVC is a starting point for exploring the impacts of methane-derived carbon solids.

The charcoal used was natural charcoal collected fresh from a forest as part of a previous study.[24] The charcoal was stored dry in the dark at 23 °C from the time of collection in 2011 to the time of this study. The carbon solids were randomly sampled, ground, and sieved to a particle size of 0.25−0.85 mm. A portion of carbon particles was washed post-production with distilled and autoclaved water to remove fine particulates and impurities[25,26] to compare the impact of washed (W) versus unwashed (UW) treatment. Particles were autoclaved at 121 °C for 2 h to remove residual DNA,[27,28] dried at room temperature, and stored dry in the dark. The properties of the ZVC and charcoal[29] are summarized in Table S1, and additional preparation details are provided in the Supporting Information.

**Bacterial Preparation and Incubation Experiment.** Model nitrogen cycling bacteria used in this study include *A. vinelandii* (ATCC 478), *N. europaea* (ATCC 19718), and *P. stutzeri* (ATCC 17588). All organisms were precultured from frozen stocks. Growth conditions can be found in the Supporting Information.

Incubation experiments to assess growth under carbon solid addition were adapted from ref 30 (growth conditions described in the Supporting Information). For washed and unwashed treatments, either nothing was added to 25 mL of medium [no treatment (NT)], 5% [by weight (1.25 g)] charcoal, or 5% (1.25 g) ZVC. Additional controls containing 5% [by weight (1.25 g)] silica sand of similar particle size were used to determine if differences were specific to carbon solids. Treatments and sampling are summarized in Tables S2 and S3. Treatments were performed in triplicate and accompanied by negative controls. Stock cultures were grown to log phase, and aliquots were added to begin bacterial growth. Aliquots were taken eight times throughout the growth experiment for samples and controls.

**Estimating Bacterial Cell Abundance.** DNA extractions and quantitative polymerase chain reaction (qPCR) targeting of the 16S rRNA gene were used to estimate bacterial abundance from incubation experiments. For each sampling time, the three replicate samples were quantified in duplicate and accompanied by negative controls. The details are available in the Supporting Information and Table S2. Standard curves were created using serial dilutions of the known gene quantity of previously amplified 16S rRNA gene PCR products. The gene copy number was calculated by comparing the threshold cycle values with the standard curve as previously described.[31]

**Bacterial Interaction with Carbon Solids.** Carbon solids were collected at the end of the incubation experiments using a 40 $\mu$m filter. Culture bottles were rinsed with sterile water several times to collect all of the solids. The solids were rinsed with sterile water several times to remove any unadhered bacteria. DNA was extracted from 0.1 g dry weight of carbon solids and sand using FastDNA Spin Kit (MP Biomedicals), and the number of adhered bacterial cells was estimated using the qPCR standard curve method as described above.

**Effects of Carbon Solids on Gene Expression.** Aliquots (500 $\mu$L) were taken during the mid log phase of bacterial growth for total RNA extraction. RT-qPCR was used to measure the expression levels of nitrogen cycling genes. The housekeeping gene *gap* and 16S rRNA were used as reference genes. For *A. vinelandii*, the transcription levels of genes involved in nitrogen fixation (*nifH*, *nifD*, *vnfD*, *anfD*, and *anfK*) and *sodC* were determined. The expressions of genes involved in nitrification (*amoA*, *amoB*, *hao1*, and *cycA*), nitrifier denitrification (*nirQ* and *norB*), and *sodB* were examined for *N. europaea*. Expressions of genes involved in denitrification (*napB*, *narG*, *nirS*, *norB*, and *nosZ*) and *sodB* were examined for *P. stutzeri*. The three replicate samples were quantified in duplicate and were accompanied by no RT and negative controls. The $2^{-\Delta\Delta Ct}$ method was used to determine relative gene expression.[32] The details are available in the Supporting Information and Table S4. Gene descriptions can be found in Table S5.

**Other Analytical Methods.** To measure the pH and nitrogen compound concentrations during bacterial growth, aliquots were spun down, and the supernatant was collected. The pH was measured using a pH meter (Thermo Fisher Scientific, Waltham, MA). Ammonium ($NH_4^+$) concentrations were quantified as previously described[33−35] using the salicylate method. Nitrite ($NO_2^-$) and nitrate ($NO_3^-$) concentrations were quantified as previously described[36,37] using the Griess method. The same methods were used to determine the adsorption of nutrients by carbon solids by measuring the pH, $NH_4^+$ concentrations, and $NO_3^-$ concentrations in the absence of bacteria at the beginning and end of the experiments.



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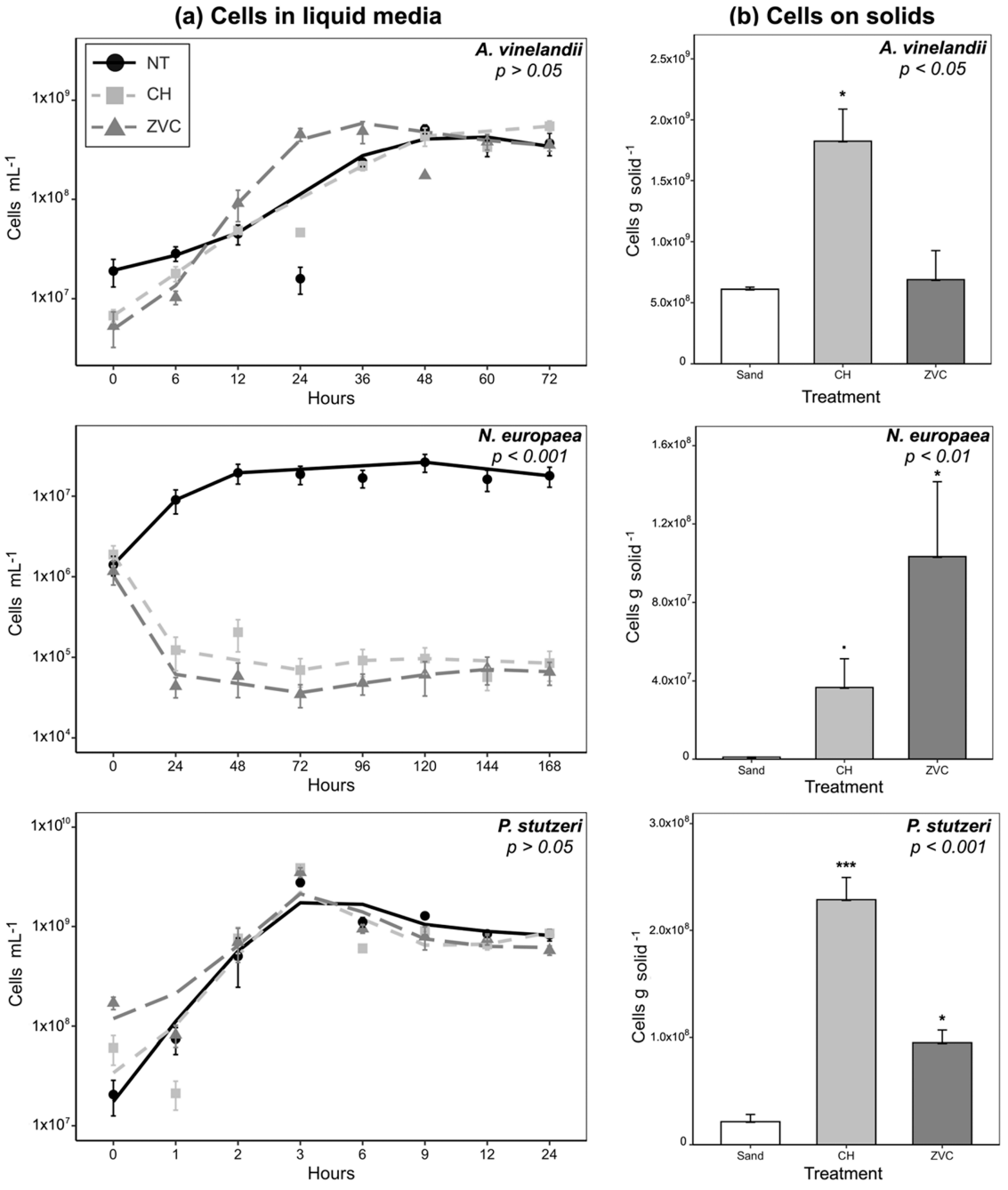


**Figure 1.** Addition of unwashed zero-valent carbon hindered growth and increased cell attachment to solids of a model ammonia oxidizer. Model nitrogen cycling bacteria were grown in the presence of unwashed 5% zero-valent carbon (ZVC), 5% charcoal (CH), or no treatment (NT). Growth curves of the (a) nitrogen fixing bacteria *A. vinelandii*, the nitrifying bacteria *N. europaea*, and the denitrifying bacteria *P. stutzeri*. (b) Bacterial cell interactions with carbon solids. Attachment per gram of solid was estimated by extraction of DNA from all solids and controls. Significant differences: •$p \leq 0.1$, *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. These values represent differences between treatments and sand control. Bars represent the standard error of three replicate samples quantified in duplicate during qPCR for panels a and b.

Statistical analysis was performed using R software.[38] Data were checked for normality and homogeneity of variance. If assumptions were met, differences between samples were determined using analysis of variance and Tukey's HSD post-hoc test. If not, differences were determined using the Kruskal–Wallis test and Dunn's post-hoc test.

## RESULTS AND DISCUSSION

**Carbon Solid Addition Had Varying Impacts on Nutrient Adsorption.** The presence of ZVC and charcoal had different impacts on the concentrations of $NH_4^+$ and $NO_3^-$ in the growth medium (Figure S1). The $NH_4^+$ concentration in the *N. europaea* growth medium was significantly reduced ($p < 0.01$) under both charcoal treatments and marginally reduced ($p < 0.1$) under ZVC-W. In *P. stutzeri* growth media, charcoal-W and charcoal-UW decreased the average $NO_3^-$ concentration from 7.9 to 6.0 and 5.4 mM, respectively. ZVC addition did not significantly decrease the $NO_3^-$ concentrations. Furthermore, the adsorption of nutrients by charcoal-W and charcoal-UW significantly decreased ($p < 0.05$) the pH of all tested media


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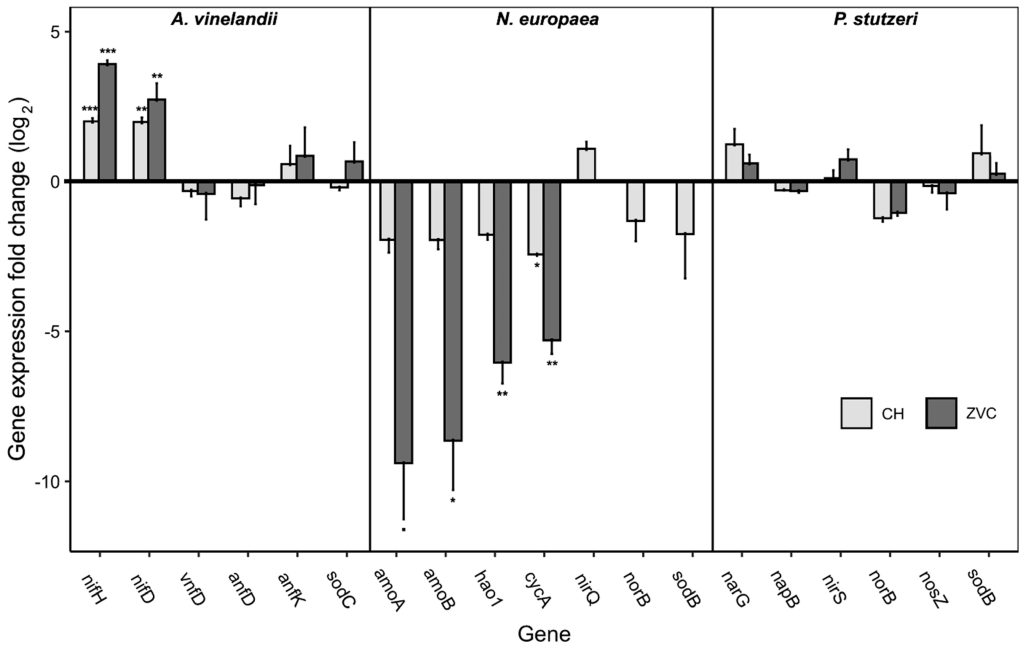


**Figure 2.** Addition of unwashed zero-valent carbon significantly down-regulated the expression of genes involved in ammonia oxidation. Model nitrogen cycling bacteria were grown in the presence of unwashed 5% zero-valent carbon (ZVC), 5% charcoal (CH), or no treatment (NT). For *N. europaea*, the genes *nirQ*, *norB*, and *sodB* were not detected under ZVC addition. Changes in gene expression were estimated using RT-qPCR and the $2^{-\Delta\Delta Ct}$ method compared to NT. Two reference genes were used, *gap* and the 16S rRNA gene. Significant differences: •$p \leq 0.1$, *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. Bars represent the standard error of three replicate samples quantified in duplicate.

(Figure S2a), while ZVC had no impact on pH. Overall, the stronger ability of charcoal to adsorb nutrients is likely related to its 50-fold larger surface area[39] and more negative surface charge (i.e., $\zeta$ potential) (Table S1).[29,40] The adsorption of $NO_3^-$ to charcoal is likely due to water containing dissolved $NO_3^-$ anchoring to the charcoal surface.[41] Therefore, the reduced surface area and greater hydrophobicity (i.e., contact angle) of ZVC may have limited $NO_3^-$ adsorption.

**Addition of Carbon Solids Differentially Impacted the Growth of Nitrogen Cycling Bacteria.** The addition of carbon solids not only changes the soil physicochemical properties but also affects the soil microbial community.[42,43] All bacteria had similar responses in growth in the presence of ZVC or charcoal (Figure 1a and Figure S3a), and sand additions had no significant impact on growth (Figure S4). The growth of *A. vinelandii* and *P. stutzeri* did not significantly ($p > 0.05$) differ under carbon solid addition compared to NT. ZVC-UW slightly increased the growth rate of *A. vinelandii*, which reached the stationary phase earlier than in the presence of charcoal or NT. Only the growth of *N. europaea* significantly decreased ($p < 0.001$) in the presence of ZVC or charcoal. Estimated cell abundance dramatically decreased after 24 h before stabilizing and resuming slight growth between 72 and 96 h. The observed differences in growth and associated metabolic activity in the presence of ZVC were reflected by changes in the pH of the medium (Figure S2b), while charcoal treatments buffered pH changes under acidic conditions.[44] Although it is unknown whether ZVC will impact all ammonia-oxidizing bacteria (AOB) similarly, *Nitrosomonas* is one of the most abundant genera of AOB in soils[45,46] and is sensitive to other carbon solid amendments.[47,48] While ammonia oxidation can be carried out by ammonia-oxidizing archaea (AOA) and comammox bacteria, AOB are generally favored in soils with high levels of fertilizers.[49−51] In such cases, inhibiting ammonia oxidation, the first and most rate-limiting step of nitrification, would be critical for restricting downstream nitrate production and denitrification.

**Nitrogen Cycling Bacteria Attached to ZVC and Charcoal.** Surface attachment is known to influence the growth and activity of microbes,[52] and all of the bacteria in this study interacted with the washed and unwashed carbon solids (Figure 1b and Figure S3b). Attachment was significantly higher ($p > 0.05$) with carbon solids than with sand. While *A. vinelandii* and *P. stutzeri* exhibited higher levels of attachment with charcoal than with ZVC, the opposite was observed for *N. europaea*. For *N. europaea*, 369- and 146-fold higher levels of attachment to ZVC-W and ZV-UW were observed compared to sand; its lower extents of attachment to CH-W and CH-UW were 84- and 51-fold higher, respectively, than to sand.

The significant attachment of *N. europaea* to carbon solids is likely responsible for the rapid decrease in the suspended cell abundance in the medium. Nitrification generally occurs at the surface of soil particles instead of in soil solution,[53] and AOB prefer to aggregate rather than exist as free-living cells.[54,55] Similar results were reported upon addition of particles like bentonite and calcium carbonate, where the majority of nitrifying bacteria attached to particles, leaving few cells in suspension.[56] While the exact mechanism for greater adhesion to ZVC is unknown, *N. europaea* may have to compete with ammonium ions for adsorption sites on charcoal similar to observations made in soils.[57] This is further supported by the greater adsorption of $NH_4^+$ to charcoal than ZVC (Figure S1), which correlated with a lower extent of cell adhesion (Figure 1b, middle panel). Moreover, increased bacterial colonization of biochar with a positive surface charge like that of ZVC has been reported.[58] Generally, bacteria attach to carbon solids because they are porous and can act as a nutrient source,[59,60] but ZVC did not adsorb a significant amount of $NH_4^+$, which could limit the activity of ZVC-attached AOB in soil environments.


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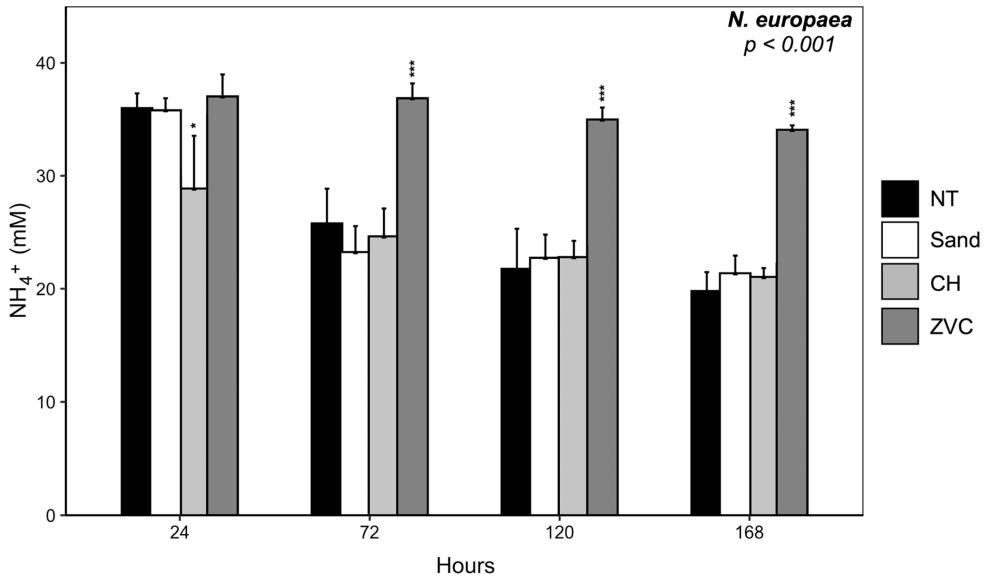


**Figure 3.** Addition of unwashed zero-valent carbon inhibited ammonium consumption by *N. europaea*. The bacterium was grown in the presence of unwashed 5% zero-valent carbon (ZVC), 5% charcoal (CH), 5% sand, or no treatment (NT). Concentrations of $NH_4^+$ in the medium over the growth period were measured. Bars represent the standard deviation of three replicate samples. Significance differences: •$p \leq 0.1$, *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. These values represent differences between treatments and no treatment.

**The Greatest Impact of ZVC Was on Ammonia Oxidation.** The biogeochemical nitrogen cycle is largely driven by functional gene expression and enzyme activities of soil microbes.[14] Thus, we examined the expression of nitrogen cycling genes and nitrogen production under carbon solid addition. ZVC addition upregulated several genes involved in nitrogen fixation (Figure 2 and Figure S5). Marginal ($p < 0.1$) or significant ($p < 0.05$) up-regulation of all nitrogenase genes occurred under ZVC-W addition, resulting in a modest increase in $NH_4^+$ production (Figure S6). This minimal to positive impact on N fixation is critical as it regulates the supply of bioavailable nitrogen in the environment.[61] In contrast, the presence of ZVC and charcoal did not significantly impact the expression of genes involved in denitrification. Most differences in gene expression were minor across treatments and did not result in a significant difference in $NO_2^-$ production (Figure S6) or $NO_3^-$ utilization.

Carbon solids had the most significant impact on nitrification. In the presence of ZVC, genes involved in ammonia oxidation (i.e., *amoA, amoB, hao1*, and *cycA*) were significantly down-regulated, while charcoal significantly down-regulated only *amoB* and *cycA*. Down-regulation was slightly greater for unwashed treatments possibly due to inhibitory residues[21] or fine carbon particulates that would otherwise be removed during washing increasing the total surface area.[62,63] The expression data were corroborated by the significantly reduced level of $NH_4^+$ consumption (Figure 3 and Figure S7) and $NO_2^-$ production ($p < 0.001$) in the presence of ZVC (Figure S6). The proportion of reduction in $NO_2^-$ production under ZVC addition was similar to that reported for chemical nitrification inhibitors.[64,65] Charcoal only resulted in a minor decrease in the level of $NO_2^-$ production, which could also be an artifact of $NO_2^-$ adsorption. No expression of genes involved in nitrifier denitrification (i.e., *nirQ* and *norB*) was detected under ZVC-W or ZVC-UW addition. These genes are associated with the removal of toxic $NO_2^-$,[45,66] which was produced at a much lower extent in these treatments (Figure S6) and likely exerted lower selective pressure for their expression. Overall, the use of ZVC as a soil amendment has the potential to reduce nitrogen emissions through direct impacts on ammonia oxidation and nitrifier denitrification.

**Environmental Implications.** Considering that microbial nitrification and denitrification are the main sources of $N_2O$ emissions from soils,[67] hindering at least one of these processes could significantly reduce the level of generation of this important greenhouse gas. While biochars generally decrease $N_2O$ emissions by impacting denitrification,[13,14] our results show that ZVC would do so primarily by inhibiting nitrification, which is the rate-limiting step controlling the ammonium:nitrate ratio in soil.[45] In this scenario, inhibition of ammonia oxidation would create a "bottleneck" in the nitrogen cycle and decrease $N_2O$ emissions by denitrifying bacteria operating downstream of nitrification.

A lower activity of ammonium oxidation could result in other positive consequences. However, further research is needed to determine how pervasive the inhibitory effect of ZVC is on other dominant nitrogen cycling bacteria, how persistent this effect would be as ZVC is transformed and "weathered" in the environment, and how effects vary depending on hydrocarbon feedstock and production conditions. Specifically, hindered nitrification would enhance N retention in soil (as $NH_4^+$), potentially enhancing plant productivity and mitigating the transformation of $NH_4^+$ to more mobile $NO_3^-$, reducing its contamination of water resources impacted by agricultural drainage. The benefits of ZVC would need to be evaluated against unintended negative consequences, such as potentially increased ammonia volatilization, which could lead to increased fine particulate aerosols,[68] as has been inferred by air quality modeling.[69] Additionally, via extrapolation from the biochar literature,[70] it is important to ensure the absence of potential associated contaminants with ZVC (e.g., heavy metals, polycyclic aromatic hydrocarbons, and environmentally persistent free radicals) that could impact the growth of soil microbes and plants and assess whether ZVC exhibits electron shuttling properties that mitigate $N_2O$ emissions.[71,72]



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Although chemical nitrification inhibitors exist for improved nutrient retention and reduced $NO_x$ and $N_2O$ emissions, their effectiveness varies and potential exposure to such residual chemicals poses health risks to animals and humans.[73] In contrast to nitrification inhibitors, ZVC could also improve soil quality similar to other carbon solids[74] and persist for a prolonged period, extending its impact on nitrification.[75] While additional studies are needed in more complex environments, ZVC as a soil amendment has the potential to serve as an alternative to chemical nitrification inhibitors and mitigate associated nitrogenous pollution, with the added benefit of long-term carbon sequestration as a carbonized material coupled with the production of clean energy.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.3c00682.

Additional details, including the properties of carbon solids (Table S1), treatments for incubation experiments (Table S2), sampling times for the incubation experiment (Table S3), primers and efficiencies (Table S4), description of primers (Table S5), nitrogen adsorption by carbon solids (Figure S1), changes in pH in the presence of carbon solids (Figure S2), impacts of washed caron solids on bacterial growth cell attachment (Figure S3), impact of sand on bacterial growth (Figure S4), impact of washed carbon solids on gene expression (Figure S5), nitrogen production by nitrogen cycling bacteria (Figure S6), and ammonium consumption in the presence of washed carbon solids (Figure S7) (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Sánchez-Bastardo, N.; Schlögl, R.; Ruland, H. Methane Pyrolysis for Zero-Emission Hydrogen Production: A Potential Bridge Technology from Fossil Fuels to a Renewable and Sustainable Hydrogen Economy. *Ind. Eng. Chem. Res.* **2021**, *60* (32), 11855−11881.

(2) Zhang, J.; Li, X.; Chen, H.; Qi, M.; Zhang, G.; Hu, H.; Ma, X. Hydrogen Production by Catalytic Methane Decomposition: Carbon Materials as Catalysts or Catalyst Supports. *Int. J. Hydrog. Energy* **2017**, *42* (31), 19755−19775.

(3) Weger, L.; Abánades, A.; Butler, T. Methane Cracking as a Bridge Technology to the Hydrogen Economy. *Int. J. Hydrog. Energy* **2017**, *42* (1), 720−731.

(4) Kavitha, B.; Reddy, P. V. L.; Kim, B.; Lee, S. S.; Pandey, S. K.; Kim, K. H. Benefits and Limitations of Biochar Amendment in Agricultural Soils: A Review. *J. Environ. Manage.* **2018**, *227*, 146−154.

(5) Ding, Y.; Liu, Y.; Liu, S.; Li, Z.; Tan, X.; Huang, X.; Zeng, G.; Zhou, L.; Zheng, B. Biochar to Improve Soil Fertility. A Review. *Agron. Sustain. Dev.* **2016**, *36* (2), 36 DOI: 10.1007/s13593-016-0372-z.

(6) Atkinson, C. J.; Fitzgerald, J. D.; Hipps, N. A. Potential Mechanisms for Achieving Agricultural Benefits from Biochar Application to Temperate Soils: A Review. *Plant Soil* **2010**, *337* (1), 1−18.

(7) Singh, B.; Singh, B. P.; Cowie, A. L. Characterisation and Evaluation of Biochars for Their Application as a Soil Amendment. *Soil Res.* **2010**, *48* (7), 516−525.

(8) Zhang, Q.; Song, Y.; Wu, Z.; Yan, X.; Gunina, A.; Kuzyakov, Y.; Xiong, Z. Effects of Six-Year Biochar Amendment on Soil Aggregation, Crop Growth, and Nitrogen and Phosphorus Use Efficiencies in a Rice-Wheat Rotation. *J. Clean. Prod.* **2020**, *242*, 118435.

(9) Zheng, Y.; Han, X.; Li, Y.; Yang, J.; Li, N.; An, N. Effects of Biochar and Straw Application on the Physicochemical and Biological Properties of Paddy Soils in Northeast China. *Sci. Rep.* **2019**, *9* (1), 16531.

(10) Bardgett, R. D.; van der Putten, W. H. Belowground Biodiversity and Ecosystem Functioning. *Nature* **2014**, *515* (7528), 505−511.

(11) Hartman, W. H.; Richardson, C. J.; Vilgalys, R.; Bruland, G. L. Environmental and Anthropogenic Controls over Bacterial Communities in Wetland Soils. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (46), 17842−17847.

(12) Cornell, C. R.; Zhang, Y.; Ning, D.; Wu, L.; Wagle, P.; Steiner, J. L.; Xiao, X.; Zhou, J. Temporal Dynamics of Bacterial Communities Along a Gradient of Disturbance in a U.S. Southern Plains Agroecosystem. *mBio* **2022**, *13* (3), e0382921.

(13) Xiao, Z.; Rasmann, S.; Yue, L.; Lian, F.; Zou, H.; Wang, Z. The Effect of Biochar Amendment on N-Cycling Genes in Soils: A Meta-Analysis. *Sci. Total Environ.* **2019**, *696*, 133984.

(14) Zhang, L.; Jing, Y.; Chen, C.; Xiang, Y.; Rezaei Rashti, M.; Li, Y.; Deng, Q.; Zhang, R. Effects of Biochar Application on Soil Nitrogen Transformation, Microbial Functional Genes, Enzyme Activity, and Plant Nitrogen Uptake: A Meta-Analysis of Field Studies. *GCB Bioenergy* **2021**, *13* (12), 1859−1873.

(15) Cayuela, M. L.; van Zwieten, L.; Singh, B. P.; Jeffery, S.; Roig, A.; Sánchez-Monedero, M. A. Biochar's Role in Mitigating Soil Nitrous Oxide Emissions: A Review and Meta-Analysis. *Agric. Ecosyst. Environ.* **2014**, *191*, 5−16.

(16) Noar, J. D.; Bruno-Bárcena, J. M. *Azotobacter vinelandii*: The Source of 100 Years of Discoveries and Many More to Come. *Microbiology* **2018**, *164* (4), 421−436.

(17) Kester, R. A.; dE Boer, W.; Laanbroek, H. J. Production of NO and $N_2O$ by Pure Cultures of Nitrifying and Denitrifying Bacteria During Changes in Aeration. *Appl. Environ. Microbiol.* **1997**, *63* (10), 3872−3877.

(18) Lovell, C. R.; Piceno, Y. M.; Quattro, J. M.; Bagwell, C. E. Molecular Analysis of Diazotroph Diversity in the Rhizosphere of the Smooth Cordgrass, Spartina Alterniflora. *Appl. Environ. Microbiol.* **2000**, *66* (9), 3814−3822.

(19) Carnol, M.; Kowalchuk, G. A.; De Boer, W. *Nitrosomonas europaea*-Like Bacteria Detected as the Dominant ß-Subclass

Proteobacteria Ammonia Oxidisers in Reference and Limed Acid Forest Soils. *Soil Biol. Biochem.* **2002**, *34* (7), 1047−1050.
(20) Lalucat, J.; Bennasar, A.; Bosch, R.; García-Valdés, E.; Palleroni, N. J. Biology of *Pseudomonas stutzeri*. *Microbiol. Mol. Biol. Rev.* **2006**, *70* (2), 510−547.
(21) Intani, K.; Latif, S.; Islam, M. S.; Müller, J. Phytotoxicity of Corncob Biochar Before and After Heat Treatment and Washing. *Sustainability* **2019**, *11* (1), 30.
(22) Xiao, X.; Chen, B. A Direct Observation of the Fine Aromatic Clusters and Molecular Structures of Biochars. *Environ. Sci. Technol.* **2017**, *51* (10), 5473−5482.
(23) Jin, H. Y.; He, Z. W.; Ren, Y. X.; Yang, W. J.; Tang, C. C.; Chen, F.; Zhou, A. J.; Liu, W.; Liang, B.; Wang, A. Role and Significance of Water and Acid Washing on Biochar for Regulating Methane Production from Waste Activated Sludge. *Sci. Total Environ.* **2022**, *817*, 152950.
(24) Pyle, L. A.; Magee, K. L.; Gallagher, M. E.; Hockaday, W. C.; Masiello, C. A. Short-Term Changes in Physical and Chemical Properties of Soil Charcoal Support Enhanced Landscape Mobility. *J. Geophys. Res. Biogeosci.* **2017**, *122* (11), 3098−3107.
(25) Karunanayake, A. G.; Todd, O. A.; Crowley, M.; Ricchetti, L. B.; Pittman, C. U.; Anderson, R.; Mlsna, T. E. Rapid Removal of Salicylic Acid, 4-Nitroaniline, Benzoic Acid and Phthalic Acid from Wastewater Using Magnetized Fast Pyrolysis Biochar from Waste Douglas Fir. *Chem. Eng. J.* **2017**, *319*, 75−88.
(26) Karunanayake, A. G.; Todd, O. A.; Crowley, M.; Ricchetti, L.; Pittman, C. U.; Anderson, R.; Mohan, D.; Mlsna, T. Lead and Cadmium Remediation Using Magnetized and Nonmagnetized Biochar from Douglas Fir. *Chem. Eng. J.* **2018**, *331*, 480−491.
(27) Gefrides, L. A.; Powell, M. C.; Donley, M. A.; Kahn, R. UV Irradiation and Autoclave Treatment for Elimination of Contaminating DNA from Laboratory Consumables. *Forensic. Sci. Int. Genet.* **2010**, *4* (2), 89−94.
(28) Trevors, J. T. Sterilization and Inhibition of Microbial Activity in Soil. *J. Microbiol. Methods* **1996**, *26* (1), 53−59.
(29) Chen, X.; Gao, X.; Yu, P.; Spanu, L.; Hinojosa, J.; Zhang, S.; Long, M.; Alvarez, P. J. J.; Masiello, C. A. Rapid Simulation of Decade-Scale Charcoal Aging in Soil: Changes in Physicochemical Properties and Their Environmental Implications. *Environ. Sci. Technol.* **2023**, *57* (1), 128−138.
(30) Hill, R. A.; Hunt, J.; Sanders, E.; Tran, M.; Burk, G. A.; Mlsna, T. E.; Fitzkee, N. C. Effect of Biochar on Microbial Growth: A Metabolomics and Bacteriological Investigation in *E. coli*. *Environ. Sci. Technol.* **2019**, *53* (5), 2635−2646.
(31) Behrens, S.; Azizian, M. F.; McMurdie, P. J.; Sabalowsky, A.; Dolan, M. E.; Semprini, L.; Spormann, A. M. Monitoring Abundance and Expression of "*Dehalococcoides*" Species Chloroethene-Reductive Dehalogenases in a Tetrachloroethene-Dechlorinating Flow Column. *Appl. Environ. Microbiol.* **2008**, *74* (18), 5695−5703.
(32) Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25* (4), 402−408.
(33) Kempers, A. J.; Kok, C. J. Re-Examination of the Determination of Ammonium as the Indophenol Blue Complex Using Salicylate. *Anal. Chim. Acta* **1989**, *221*, 147−155.
(34) Lee, J. G.; Chae, H. G.; Hwang, H. Y.; Kim, P. J.; Cho, S. R. Effect of Plastic Film Mulching on Maize Productivity and Nitrogen Use Efficiency under Organic Farming in South Korea. *Sci. Total Environ.* **2021**, *787*, 147503.
(35) Klotz, F.; Kitzinger, K.; Ngugi, D. K.; Büsing, P.; Littmann, S.; Kuypers, M. M. M.; Schink, B.; Pester, M. Quantification of Archaea-Driven Freshwater Nitrification from Single Cell to Ecosystem Levels. *ISME J.* **2022**, *16* (6), 1647−1656.
(36) Granger, D. L.; Taintor, R. R.; Boockvar, K. S.; Hibbs, J. J. B. Determination of Nitrate and Nitrite in Biological Samples Using Bacterial Nitrate Reductase Coupled with the Griess Reaction. *Methods* **1995**, *7* (1), 78−83.
(37) McNeely, K.; Kumaraswamy, G. K.; Guerra, T.; Bennette, N.; Ananyev, G.; Dismukes, G. C. Metabolic Switching of Central Carbon Metabolism in Response to Nitrate: Application to Autofermentative Hydrogen Production in Cyanobacteria. *J. Biotechnol.* **2014**, *182*−*183*, 83−91.
(38) R Core Team. *R: A Language and Environment for Statistical Computing*; Vienna, 2020 (https://www.r-project.org/).
(39) Zeng, Z.; Zhang, S. D.; Li, T. Q.; Zhao, F. L.; He, Z. L.; Zhao, H. P.; Yang, X. E.; Wang, H. L.; Zhao, J.; Rafiq, M. T. Sorption of Ammonium and Phosphate from Aqueous Solution by Biochar Derived from Phytoremediation Plants. *J. Zhejiang Univ. Sci. B* **2013**, *14* (12), 1152−1161.
(40) Mukherjee, A.; Zimmerman, A. R.; Harris, W. Surface Chemistry Variations among a Series of Laboratory-Produced Biochars. *Geoderma* **2011**, *163* (3), 247−255.
(41) Hagemann, N.; Joseph, S.; Schmidt, H.-P.; Kammann, C. I.; Harter, J.; Borch, T.; Young, R. B.; Varga, K.; Taherymoosavi, S.; Elliott, K. W.; McKenna, A.; Albu, M.; Mayrhofer, C.; Obst, M.; Conte, P.; Dieguez-Alonso, A.; Orsetti, S.; Subdiaga, E.; Behrens, S.; Kappler, A. Organic Coating on Biochar Explains Its Nutrient Retention and Stimulation of Soil Fertility. *Nat. Commun.* **2017**, *8* (1), 1089.
(42) Zhang, L.; Jing, Y.; Xiang, Y.; Zhang, R.; Lu, H. Responses of Soil Microbial Community Structure Changes and Activities to Biochar Addition: A Meta-Analysis. *Sci. Total Environ.* **2018**, *643*, 926−935.
(43) Muhammad, N.; Dai, Z.; Xiao, K.; Meng, J.; Brookes, P. C.; Liu, X.; Wang, H.; Wu, J.; Xu, J. Changes in Microbial Community Structure Due to Biochars Generated from Different Feedstocks and Their Relationships with Soil Chemical Properties. *Geoderma* **2014**, *226*−*227*, 270−278.
(44) Zhu, X.; Chen, B.; Zhu, L.; Xing, B. Effects and Mechanisms of Biochar-Microbe Interactions in Soil Improvement and Pollution Remediation: A Review. *Environ. Pollut.* **2017**, *227*, 98−115.
(45) Kowalchuk, G. A.; Stephen, J. R. Ammonia-Oxidizing Bacteria: A Model for Molecular Microbial Ecology. *Annu. Rev. Microbiol.* **2001**, *55* (1), 485−529.
(46) Jiang, H.; Huang, L.; Deng, Y.; Wang, S.; Zhou, Y.; Liu, L.; Dong, H. Latitudinal Distribution of Ammonia-Oxidizing Bacteria and Archaea in the Agricultural Soils of Eastern China. *Appl. Environ. Microbiol.* **2014**, *80* (18), 5593−5602.
(47) Liu, Z.; Shang, H.; Han, F.; Zhang, M.; Li, Q.; Zhou, W. Improvement of Nitrogen and Phosphorus Availability by *Pseudoalteromonas* sp. During Salt-Washing in Saline-Alkali Soil. *Appl. Soil Ecol.* **2021**, *168*, 104117.
(48) Yao, R. J.; Li, H. Q.; Yang, J. S.; Wang, X. P.; Xie, W. P.; Zhang, X. Biochar Addition Inhibits Nitrification by Shifting Community Structure of Ammonia-Oxidizing Microorganisms in Salt-Affected Irrigation-Silting Soil. *Microorganisms* **2022**, *10* (2), 436.
(49) Di, H. J.; Cameron, K. C.; Shen, J. P.; Winefield, C. S.; O'Callaghan, M.; Bowatte, S.; He, J. Z. Ammonia-Oxidizing Bacteria and Archaea Grow under Contrasting Soil Nitrogen Conditions. *FEMS Microbiol. Ecol.* **2010**, *72* (3), 386−394.
(50) Verhamme, D. T.; Prosser, J. I.; Nicol, G. W. Ammonia Concentration Determines Differential Growth of Ammonia-Oxidising Archaea and Bacteria in Soil Microcosms. *ISME J.* **2011**, *5* (6), 1067−1071.
(51) Hink, L.; Nicol, G. W.; Prosser, J. I. Archaea Produce Lower Yields of $N_2O$ than Bacteria During Aerobic Ammonia Oxidation in Soil. *Environ. Microbiol.* **2017**, *19* (12), 4829−4837.
(52) Bright, J. J.; Fletcher, M. Amino Acid Assimilation and Electron Transport System Activity in Attached and Free-Living Marine Bacteria. *Appl. Environ. Microbiol.* **1983**, *45* (3), 818−825.
(53) Underhill, S. E.; Prosser, J. I. Surface Attachment of Nitrifying Bacteria and Their Inhibition by Potassium Ethyl Xanthate. *Microb. Ecol.* **1987**, *14* (2), 129−139.
(54) Allison, S. M.; Prosser, J. I. Ammonia Oxidation at Low pH by Attached Populations of Nitrifying Bacteria. *Soil Biol. Biochem.* **1993**, *25* (7), 935−941.
(55) Vejmelkova, D.; Sorokin, D. Y.; Abbas, B.; Kovaleva, O. L.; Kleerebezem, R.; Kampschreur, M. J.; Muyzer, G.; van Loosdrecht, M. C. Analysis of Ammonia-Oxidizing Bacteria Dominating in Lab-Scale


https://doi.org/10.1021/acs.estlett.3c00682
*Environ. Sci. Technol. Lett.* 2023, 10, 1082−1089

Bioreactors with High Ammonium Bicarbonate Loading. *Appl. Microbiol. Biotechnol.* **2012**, *93* (1), 401−410.
(56) Diab, S.; Shilo, M. Effect of Adhesion to Particles on the Survival and Activity of *Nitrosomonas* sp. and *Nitrobacter* sp. *Arch. Microbiol.* **1988**, *150* (4), 387−393.
(57) Lees, H.; Quastel, J. H. Biochemistry of Nitrification in Soil: 2. The Site of Soil Nitrification. *Biochem. J.* **1946**, *40* (5−6), 815−823.
(58) Liu, Y.; Liu, S.; Yang, Z.; Xiao, L. Synergetic Effects of Biochars and Denitrifier on Nitrate Removal. *Bioresour. Technol.* **2021**, *335*, 125245.
(59) Zhao, L.; Xiao, D.; Liu, Y.; Xu, H.; Nan, H.; Li, D.; Kan, Y.; Cao, X. Biochar as Simultaneous Shelter, Adsorbent, pH Buffer, and Substrate of Pseudomonas Citronellolis to Promote Biodegradation of High Concentrations of Phenol in Wastewater. *Water Res.* **2020**, *172*, 115494.
(60) Bolan, S.; Hou, D.; Wang, L.; Hale, L.; Egamberdieva, D.; Tammeorg, P.; Li, R.; Wang, B.; Xu, J.; Wang, T.; Sun, H.; Padhye, L. P.; Wang, H.; Siddique, K. H. M.; Rinklebe, J.; Kirkham, M. B.; Bolan, N. The Potential of Biochar as a Microbial Carrier for Agricultural and Environmental Applications. *Sci. Total Environ.* **2023**, *886*, 163968.
(61) Jetten, M. S. M. The Microbial Nitrogen Cycle. *Environ. Microbiol.* **2008**, *10* (11), 2903−2909.
(62) Xie, T.; Reddy, K. R.; Wang, C.; Yargicoglu, E.; Spokas, K. Characteristics and Applications of Biochar for Environmental Remediation: A Review. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45* (9), 939−969.
(63) Alghamdi, A. G.; Alkhasha, A.; Ibrahim, H. M. Effect of Biochar Particle Size on Water Retention and Availability in a Sandy Loam Soil. *J. Saudi Chem. Soc.* **2020**, *24* (12), 1042−1050.
(64) Takagi, S.; Murakami, M.; Sato, Y.; Takahashi, R.; Tokuyama, T.; Wakabayashi, K. Effect of Nitrification Inhibitors on *Nitrosomonas* and *Nitrobacter*. *J. Pestic. Sci.* **1994**, *19* (1), 19−23.
(65) Zacherl, B.; Amberger, A. Effect of the Nitrification Inhibitors Dicyandiamide, Nitrapyrin and Thiourea on *Nitrosomonas europaea*. *Fertil. Res.* **1990**, *22*, 37−44.
(66) Poth, M.; Focht, D. D. N Kinetic Analysis of $N_2O$ Production by *Nitrosomonas europaea*: An Examination of Nitrifier Denitrification. *Appl. Environ. Microbiol.* **1985**, *49* (5), 1134−1141.
(67) Braker, G.; Conrad, R. Diversity, Structure, and Size of $N_2O$-Producing Microbial Communities in Soils—What Matters for Their Functioning? *Adv. Appl. Microbiol.* **2011**, *75*, 33−70.
(68) Qiao, C.; Liu, L.; Hu, S.; Compton, J. E.; Greaver, T. L.; Li, Q. How Inhibiting Nitrification Affects Nitrogen Cycle and Reduces Environmental Impacts of Anthropogenic Nitrogen Input. *Glob. Change Biol.* **2015**, *21* (3), 1249−1257.
(69) Luo, L.; Ran, L.; Rasool, Q. Z.; Cohan, D. S. Integrated Modeling of U.S. Agricultural Soil Emissions of Reactive Nitrogen and Associated Impacts on Air Pollution, Health, and Climate. *Environ. Sci. Technol.* **2022**, *56* (13), 9265−9276.
(70) Xiang, L.; Liu, S.; Ye, S.; Yang, H.; Song, B.; Qin, F.; Shen, M.; Tan, C.; Zeng, G.; Tan, X. Potential Hazards of Biochar: The Negative Environmental Impacts of Biochar Applications. *J. Hazard. Mater.* **2021**, *420*, 126611.
(71) Yuan, H.; Zhang, Z.; Li, M.; Clough, T.; Wrage-Mönnig, N.; Qin, S.; Ge, T.; Liao, H.; Zhou, S. Biochar's Role as an Electron Shuttle for Mediating Soil $N_2O$ Emissions. *Soil Biol. Biochem.* **2019**, *133*, 94−96.
(72) Wu, P.; Xie, M.; Clough, T. J.; Yuan, D.; Wu, S.; He, X.; Hu, C.; Zhou, S.; Qin, S. Biochar-Derived Persistent Free Radicals and Reactive Oxygen Species Reduce the Potential of Biochar to Mitigate Soil $N_2O$ Emissions by Inhibiting *nosZ*. *Soil Biol. Biochem.* **2023**, *178*, 108970.
(73) Woodward, E. E.; Edwards, T. M.; Givens, C. E.; Kolpin, D. W.; Hladik, M. L. Widespread Use of the Nitrification Inhibitor Nitrapyrin: Assessing Benefits and Costs to Agriculture, Ecosystems, and Environmental Health. *Environ. Sci. Technol.* **2021**, *55* (3), 1345−1353.
(74) Novak, J. M.; Cantrell, K. B.; Watts, D. W.; Busscher, W. J.; Johnson, M. G. Designing Relevant Biochars as Soil Amendments Using Lignocellulosic-Based and Manure-Based Feedstocks. *J. Soils Sediments* **2014**, *14* (2), 330−343.
(75) Allohverdi, T.; Mohanty, A. K.; Roy, P.; Misra, M. A Review on Current Status of Biochar Uses in Agriculture. *Molecules* **2021**, *26* (18), 5584.


https://doi.org/10.1021/acs.estlett.3c00682
*Environ. Sci. Technol. Lett.* 2023, 10, 1082−1089