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Fire-derived organic matter retains ammonia through covalent bond formation

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Fire-derived organic matter, often referred to as pyrogenic organic matter (PyOM), is present in the Earth's soil, sediment, atmosphere, and water. We investigated interactions of PyOM with ammonia (NH₃) gas, which makes up much of the Earth's reactive nitrogen (N) pool. Here we show that PyOM's NH₃ retention capacity under ambient conditions can exceed 180 mg N g⁻¹ PyOM-carbon, resulting in a material with a higher N content than any unprocessed plant material and most animal manures. As PyOM is weathered, NH₃ retention increases sixfold, with more than half of the N retained through chemisorption rather than physisorption. Near-edge X-ray absorption fine structure and nuclear magnetic resonance spectroscopy reveal that a variety of covalent bonds form between NH₃-N and PyOM, more than 10% of which contained heterocyclic structures. We estimate that through these mechanisms soil PyOM stocks could retain more than 600-fold annual NH₃ emissions from agriculture, exerting an important control on global N cycling.

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The Earth's soil, atmosphere, marine sediment, and ocean water contain large quantities of pyrogenic C (54–109, 0.26–10⁻³, 480–1440, and 26–145 Pg of C, respectively^{1,2}). In soil, most of this pyrogenic C originates from burnt biomass generated during vegetation fires, which contributes up to 129 Tg yr⁻¹ of PyOM–carbon (PyOM–C) to soil C stocks¹. Many aspects of pyrogenic C biogeochemistry remain poorly understood, including interactions between pyrogenic C's heterogeneous surface—containing both aromatic and aliphatic C, condensates, and other elements such as N, H, and O—and environmental N sources. Interactions between PyOM and environmental N may influence gaseous N emissions, N leaching, N availability to living organisms, and global N transport³. Here, we focus on PyOM's interactions with NH₃—the atmosphere's most abundant alkaline gas. Global NH₃ emissions are projected to double by 2050 and constitute a large part of the Earth's reactive N pool^{4–6}. Common sources of NH₃ in soils include decomposing organic matter, rainwater, and N fertilizer. Laboratory studies show that various forms of natural and industrially modified organic matter can retain NH₃^{7–18}, but the NH₃ retention capacity of natural PyOM stocks and the mechanisms responsible for NH₃ retention under ambient conditions have not been established. Therefore, the extent to which these studies can inform our understanding of PyOM's role in global biogeochemical cycles is unknown.

Proposed mechanisms for NH₃ retention by natural PyOM include physisorption, electrostatic interactions, and precipitation of ammonium (NH₄⁺) salts^{7–9}. Although these retention mechanisms would allow PyOM to act as a temporary N sink, N retained in these ways would be readily available for plant and microbial uptake, or loss through gas or solute transport⁷. Conversely, the formation of stronger covalent bonds between PyOM and NH₃ would result in more persistent N retention, allowing PyOM to serve as a dynamic, long-term N source and sink—both capturing NH₃ from its surroundings and slowly releasing it over time. This could also result in greater coupling of global C and N cycling, as covalently bound NH₃–N would be carried with the PyOM–C as it traveled over great distances¹. However, until now, covalent bond formation between natural PyOM and NH₃ under ambient terrestrial conditions has not been observed.

Some evidence exists that under certain laboratory conditions, covalent bonds can form between NH₃ and industrially produced relatives of PyOM or secondary organic aerosols found in the atmosphere. Graphene oxides and activated carbons can form a variety of cyclic and non-cyclic N structures when exposed to NH₃ at temperatures exceeding 200 °C^{10–14,17,18}. However, these materials are often modified (e.g., through exposure to chemical oxidants and heat or impregnated with metals), differ considerably from natural PyOM in surface area and functional group composition, and are exposed to NH₃ under conditions that are not representative of the natural environment^{19–21}. Thus, it is unknown whether these studies of graphene oxides and activated carbons can be used to predict interactions between natural PyOM and NH₃, and whether the same variety of covalent N structures would develop under natural environmental conditions. Following exposure to NH₃ at ambient temperatures, industrial relatives of PyOM can form non-cyclic amine and amide bonds²². Secondary organic aerosols found in the atmosphere—which contain functional groups present in terrestrial PyOM—can form both non-cyclic N structures as well as N heterocycles following exposure to NH₃, NH₄⁺, and amino acids under atmospheric conditions^{23,24}. However, the formation of aromatic and non-aromatic heterocyclic N structures between terrestrial PyOM and NH₃ has never been observed under ambient environmental conditions. This is of great interest because enrichment with these heterocyclic N structures

influences the electrochemical properties, absorptive capacity, and environmental persistence of both natural and industrial pyrogenic C materials^{16,22–27}. If heterocyclic N structures develop between PyOM and NH₃ under natural conditions, this interaction would have important consequences for global C and N cycling.

In order to assess the impact of PyOM stocks on global nutrient cycles, it is also necessary to consider PyOM's dynamic nature. Similar to the variety found in other sources of organic matter, different types of PyOM have different physical and chemical characteristics, including elemental makeup, functional group composition, surface area, pH, and other properties²⁸. Additionally, PyOM properties change over time, as the material is exposed to water, sunlight, microbial activity, and other oxidizing forces^{29–31}. Such variation in physiochemical properties can drastically alter PyOM's role in the environment. Thus, to understand the influence of PyOM–NH₃ interactions on global N cycling, it is important to consider how PyOM's NH₃ retention capacity might change over time. In this study, we investigate PyOM's NH₃ retention capacity under ambient conditions, N retention mechanisms, and whether retention capacity develops as PyOM stocks are weathered. We find that PyOM retains a surprising quantity of NH₃–N and that this retention capacity increases significantly as PyOM is exposed to conditions mimicking natural weathering processes. More than half of the NH₃–N is retained through chemisorption, including the formation of a variety of covalent bonds. We estimate that through these mechanisms soil PyOM stocks could play an important role in the global N cycle.

Results

Weathering increases PyOM N retention capacity. PyOM produced from woody biomass was oxidized to generate a gradient of weathered PyOM^{30,31} and subsequently exposed to NH₃ vapor at ambient temperature and pressure (35 °C and 80–800 Torr). Total NH₃ capture increased more than sixfold after oxidation, from 2.3 mmol g⁻¹ PyOM–C in unoxidized PyOM to 13.5 mmol g⁻¹ PyOM–C in highly oxidized PyOM (Fig. 1a), showing that PyOM can retain substantial quantities of N from this form of NH₃. Although specific surface area (SSA) and low pH may contribute to the NH₃ retention capacity of some pyrogenic C materials^{7,8,32}, these characteristics did not explain the trends observed here. PyOM SSA decreased with oxidation and therefore could not have contributed to the increase in NH₃ retention observed in highly oxidized PyOM samples (Fig. 1b). PyOM pH also decreased with oxidation (Fig. 1c). However, when unoxidized PyOM was incubated with hydrochloric acid, which lowered its pH without altering key oxygen-containing functional groups (Supplementary Fig. 1), NH₃ retention remained unchanged (Supplementary Fig. 2). This shows that although oxidation and low pH are correlated, pH itself did not drive NH₃ retention and cannot be used to predict PyOM oxidation or NH₃ retention capacity. Instead, our analyses indicate that functional group composition may be a more reliable determinant of PyOM's NH₃ retention capacity^{11,21}. Peak height ratios measured by Fourier transform infrared spectroscopy (FTIR) and integrated peak areas measured by solid-state ¹³C nuclear magnetic resonance (NMR) spectroscopy suggest that with progressive oxidation, PyOM's oxygen-containing functional groups increase relative to aromatic C structures (Fig. 1d and Supplementary Figs. 3 and 4). Quantitative stoichiometric measurements show that an increase in PyOM O:C ratio corresponds with the same trends observed through these spectral analyses. Taken together, these results highlight PyOM's substantial and dynamic N retention capacity, and the relevance of weathering

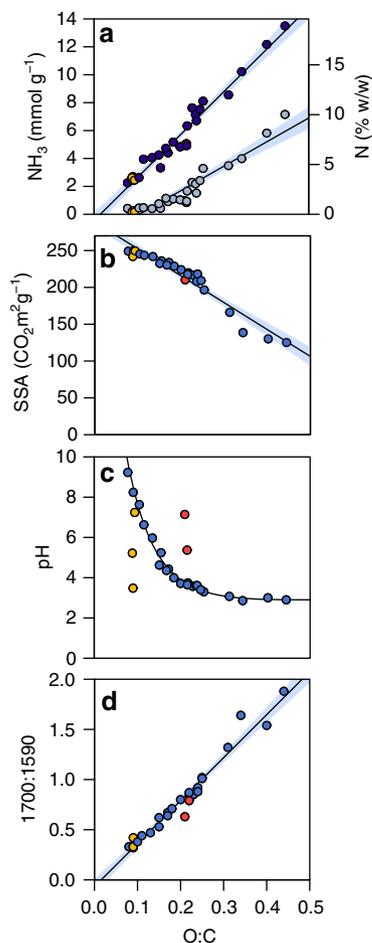


Fig. 1 Changes in pyrogenic organic matter ammonia retention and physiochemical characteristics as a function of molar O:C ratio. **a** ammonia (NH_3) retention capacity—expressed in $\text{mmol of NH}_3 \text{ g}^{-1}$ of pyrogenic organic matter-carbon (PyOM-C, left y axis) and percent nitrogen (N) of PyOM-C (right y axis)—increases as a function of molar O:C ratio. Each point represents the average oxygen:carbon (O:C) ratio for two replicates. NH_3 chemisorption = $17.49x - 1.84$, $R^2 = 0.89$, $p < 0.001$, $F_{1,25} = 204.7$, $S_{25} = 0.59$ (light blue); NH_3 combined chemical and physical adsorption = $30.51x - 0.44$, $R^2 = 0.96$, $p < 0.001$, $F_{1,25} = 567.2$, $S_{25} = 0.62$ (dark blue). **b** Specific surface area (SSA) decreases as PyOM O:C ratio increases. $\text{SSA} = -365x + 288.8$, $R^2 = 0.931$, $p < 0.001$, $F_{1,25} = 338.5$, $S_{25} = 9.591$. **c** PyOM pH decreases as oxidation increases. Blue symbols represent unoxidized PyOM and PyOM incubated with deionized water (DIH_2O) and hydrogen peroxide (H_2O_2) and are fitted with a significant curve ($y = 20.8 \cdot e^{-14.8(\text{O:C})} + 2.84$, $S_{19} = 0.199$). **d** The intensity of Fourier transform infrared (FTIR) peak heights associated with C=O stretching ($1691\text{--}1715 \text{ cm}^{-1}$) increases in proportion to the intensity of peak heights associated with C=C vibrations and stretching ($1582\text{--}1609 \text{ cm}^{-1}$) as PyOM O:C ratio increases ($y = 4.29x - 0.0670$, $R^2 = 0.963$, $p < 0.001$, $F_{1,25} = 650$, $S_{25} = 0.081$). For all figures, yellow symbols represent PyOM that was incubated with 1 M hydrochloric acid (HCl); pink symbols represent PyOM that was incubated with H_2O_2 and then with 1 M sodium hydroxide (NaOH); shaded bands represent the 95% confidence intervals

and exposure to oxidizing agents (e.g., microbial activity or ozone) when considering PyOM's potential role in N cycling.

PyOM retains $\text{NH}_3\text{-N}$ through chemisorption. In addition to revealing PyOM's considerable NH_3 retention capacity, adsorption isotherms showed that up to 53% of the NH_3 was retained

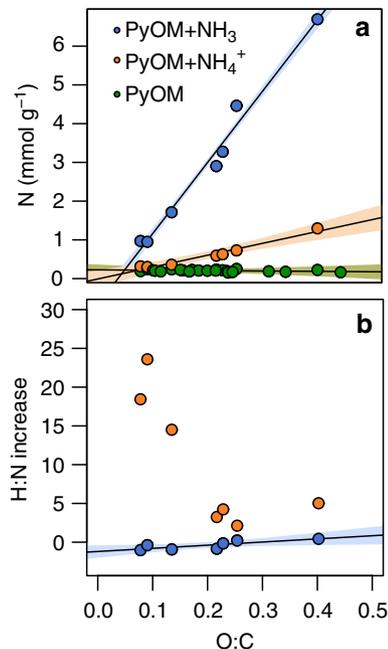


Fig. 2 Pyrogenic organic matter N content and H:N ratio following exposure to ammonium or ammonia. **a** Pyrogenic organic matter (PyOM) nitrogen (N) retention in mmol g^{-1} PyOM-carbon (C) measured by dry combustion is significantly associated with molar O:C ratios following exposure to NH_3 and NH_4^+ ($y = 18.25x - 0.0665$, $p < 0.001$ and $y = 3.045x - 0.008$, $p < 0.001$, respectively). **b** H:N molar increases are significantly associated with O:C ratios following exposure to NH_3 ($y = 6.23x - 1.70$, $R^2 = 0.59$, $p < 0.005$, $F_{1,10} = 14.45$, $\text{RSE}_{10} = 0.53$), but not NH_4^+ . PyOM samples retain < 0.5 moles of $\text{NH}_3\text{-H}$ for every mole of $\text{NH}_3\text{-N}$ retained, compared to 2.13–23.60 moles of $\text{NH}_4^+\text{-H}$ for every mole of $\text{NH}_4^+\text{-N}$ retained. Green symbols represent original PyOM samples without N addition, orange symbols represent PyOM following exposure to NH_4^+ , and blue symbols represent PyOM following exposure to NH_3 . Shaded bands represent the 95% confidence intervals

through chemisorption rather than physisorption, and that this proportion was greatest in oxidized PyOM (Fig. 1a). A commonly proposed mechanism for NH_3 chemisorption by PyOM is protonation of NH_3 to form NH_4^+ and subsequent electrostatic interaction between the NH_4^+ and PyOM's negatively-charged functional groups⁷, but our data indicate that this mechanism cannot solely be responsible for PyOM $\text{NH}_3\text{-N}$ retention. Direct exposure of oxidized PyOM to NH_4^+ resulted in much lower N retention than exposure to NH_3 gas (Fig. 2a), suggesting that electrostatic interactions alone cannot explain PyOM's NH_3 retention capacity. Furthermore, if physisorption or electrostatic interactions are predominantly responsible for PyOM-N retention from NH_3 and NH_4^+ , then stoichiometry dictates that on a molar basis, increases in PyOM-N following exposure should be accompanied by a threefold to fourfold increase in PyOM-H. However, when oxidized PyOM (molar O:C ratio 0.402) was exposed to NH_3 , the increase in PyOM molar H:N ratio was smaller than 0.5, suggesting that a substantial portion of $\text{NH}_3\text{-N}$ is retained without retention of $\text{NH}_3\text{-H}$ (Fig. 2b). When the same PyOM sample was exposed to NH_4^+ , the molar H:N ratio increased by 5.03, suggesting that most of the $\text{NH}_4^+\text{-N}$ was retained along with NH_4^+ 's H atoms. This stoichiometric comparison of H:N ratios in PyOM samples before and after exposure to NH_3 and NH_4^+ indicates that the respective mechanisms for N retention differ substantially, and that alternatives to physisorption and electrostatic interaction are likely responsible for PyOM's NH_3 retention capacity.

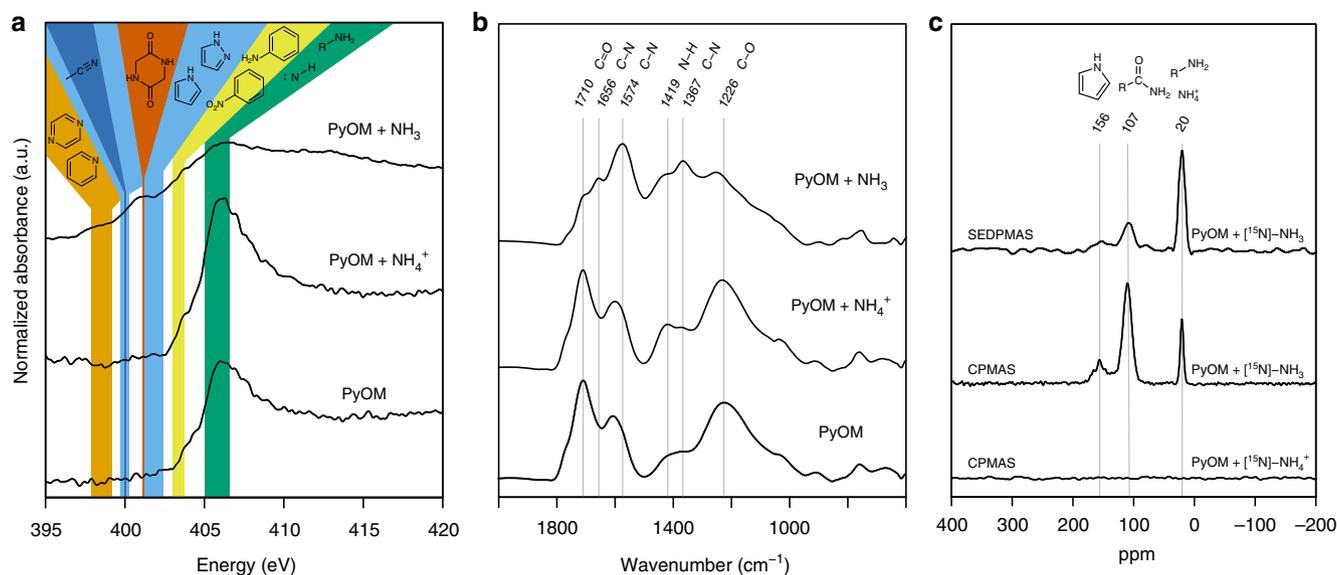


Fig. 3 Nitrogen K-edge NEXAFS, FTIR, and NMR spectra of oxidized PyOM samples. Nitrogen (N) K-edge near-edge X-ray absorption fine structure (NEXAFS) (**a**), Fourier transform infrared (FTIR) (**b**), and nuclear magnetic resonance (NMR) (**c**) spectra collected from oxidized PyOM, oxidized PyOM following exposure to ammonium (NH₄⁺), and oxidized pyrogenic organic matter (PyOM) following exposure to ammonia (NH₃). **a** Shaded bands represent the range of peak centers consistent with selected spectral features: 397.88–399.20 eV for C=N bonds in 1N and 2N aromatic six-membered rings (orange), 400.00 for nitrile bonds (dark blue), 399.76–400.27 for C=N bonds in 2N five-membered rings (light blue), 401.15 for C-N bonds in non-aromatic six-membered rings (red), 401.20–402.40 for C-N bonds in 1N and 2N aromatic five-membered rings (light blue), 403.00–403.75 for aliphatic N bonded to aromatic rings (yellow), and 405.00–406.58 for aliphatic amines and N-H bonds (green). Model chemical structures are shown at the top of the figure. **b** FTIR spectra of oxidized PyOM, oxidized PyOM following exposure to NH₄⁺, and oxidized PyOM following exposure to NH₃. **c** ¹⁵N-NMR spin echo direct polarization magic angle spinning (SEDPMAS) spectrum of oxidized PyOM following exposure to [¹⁵N]-NH₃, and ¹⁵N-NMR cross-polarization magic angle spinning (CPMAS) spectra of oxidized PyOM following exposure to [¹⁵N]-NH₃ and [¹⁵N]-NH₄⁺. The spectra suggest that NH₃-N retention mechanisms could include NH₄⁺ adsorption (represented by the peak at 20 ppm), and the formation of covalent C-N bonds such as amines (20 ppm), amides (~107 ppm), and aromatic five-membered heterocycles (chemical shifts between ~130–165 ppm)^{16,33,39,40}

PyOM and NH₃-N form covalent bonds. To examine these alternative mechanisms for NH₃ retention, we compared the N near-edge X-ray absorption fine structure (*K*-edge NEXAFS) spectra of oxidized PyOM (O:C ratio 0.402) to those of oxidized PyOM following exposure to either NH₃ or NH₄⁺ (Fig. 3a and Supplementary Fig. 5). This method cannot be used to quantify the absolute amount of N retained by PyOM, but does provide information about the types of covalent N bonds present. Exposure to NH₃ resulted in the formation of a variety of covalent N bonds that differ from those originating from PyOM feedstock N, N structures formed during thermal decomposition, N structures formed between PyOM and NH₄⁺, and N-H bonds in pure NH₃ or NH₄⁺^{33–36}. Interactions between oxidized PyOM and NH₃ led to the strong development of absorption peaks between 397.88 and 402.40 eV, many of which are consistent with aromatic and non-aromatic heterocyclic N structures^{14,36} (see Supplementary Tables 1–3). Compared to spectra collected from PyOM that was not exposed to additional N, spectra collected from PyOM following exposure to NH₃ showed a threefold increase in the $1s \rightarrow \pi^*$ area consistent with aromatic six-membered heterocycles containing either one or two N atoms (model peaks located at 397.88, 398.76, and 399.2 eV), a threefold increase in the area consistent with nitrile bonds and aromatic five-membered heterocycles containing either one or two N atoms (400.05, 401.43, and 402.40 eV), and a 1.3-fold increase in the area consistent with aliphatic N bonded to aromatic rings (403.00 and 403.65 eV) (Supplementary Table 2). Additionally, while no feature consistent with non-aromatic six-membered N heterocycles (401.15 eV) was identified in spectra collected from oxidized PyOM not exposed to NH₃, this feature accounted for two percent of the area underneath the spectrum collected from oxidized PyOM following exposure to NH₃. In contrast, development of

peaks in the $1s \rightarrow \pi^*$ region following exposure to NH₄⁺ was very small, suggesting that there was little change in N functional group composition with NH₄⁺ addition. Both the spectra of PyOM exposed to NH₄⁺ as well as those of unexposed PyOM are strongly dominated by $1s \rightarrow \sigma^*$ features with peak centers between 405.00 and 406.58 eV, which dwarf the $1s \rightarrow \pi^*$ region area in these spectra. Although these $1s \rightarrow \sigma^*$ features are often associated with N-H bonds, they cannot be assigned definitively due to severe overlap of spectral features in this region³⁶.

To further compare mechanisms for PyOM retention of NH₃ in comparison to NH₄⁺, we also collected FTIR spectra from oxidized PyOM, and oxidized PyOM following exposure to NH₃ and NH₄⁺ (Fig. 3b). Similar to the NEXAFS spectra (Fig. 3a), the FTIR spectra show clear differences between functional groups present in PyOM, PyOM following exposure to NH₃, and PyOM following exposure to NH₄⁺. Exposure to NH₃ resulted in the emergence of new peaks at 1656 and 1367 cm⁻¹ and an increase in peak height at 1574 cm⁻¹, all of which are consistent with C-N stretching, including C-N resonance stretching in aromatic rings at 1656 cm⁻¹^{37,38}. Exposure to NH₃ also resulted in a marked decrease in the peaks associated with C=O and C-O carbonyl/carboxyl and ketonic stretching at 1710 and 1226 cm⁻¹, respectively, suggesting that these functional groups decrease relative to other functional groups in this sample. In contrast, exposure to NH₄⁺ resulted in only two new spectral features, medium-sized peaks at 1419 and 1372 cm⁻¹, which are consistent with N-H and C-N stretching, respectively.

Definitive functional group assignment using FTIR spectra collected from heterogeneous materials such as PyOM is challenging because the regions associated with different bonds often overlap with one another. However, the major treatment difference between our PyOM samples is whether or not they

were exposed to gaseous NH_3 or aqueous NH_4^+ . Therefore, although FTIR spectral features between 1200 and 1700 cm^{-1} are sometimes associated with bonds between other elements (including C and O), it is probable that the emergence of distinct features in the FTIR spectra collected from our oxidized PyOM samples following exposure to either NH_3 or NH_4^+ is a result of bonds that formed between PyOM and N. Since the FTIR spectrum of pure NH_3 contains predominant peaks around 950 cm^{-1} , it is also unlikely that physical adsorption of NH_3 alone could account for the differences observed between the FTIR spectra collected from our PyOM samples before and after NH_3 exposure³⁸. In contrast, the FTIR spectra of NH_4^+ standards contain predominant peaks around 1440 cm^{-1} , indicating that NH_4^+ adsorption was responsible for the relative increase in this region of the spectra collected from our PyOM samples following exposure to NH_4^+ . This is consistent with our NEXAFS deconvolution analysis, which shows that exposure to NH_4^+ does not result in the substantial formation of a variety of N functional groups, despite the increased N content relative to unexposed PyOM samples. It also is consistent with elemental analyses, which suggest that oxidized PyOM retains NH_4^+ -N in stoichiometric balance with NH_4^+ -H, but retains NH_3 -N without NH_3 -H.

To further investigate mechanisms for NH_3 -N and NH_4^+ -N retention, we collected solid-state ^{15}N -NMR spectra after separate exposure of oxidized PyOM to enriched ^{15}N - NH_3 gas or ^{15}N - NH_4^+ solution (Fig. 3c). Use of ^{15}N -enriched reagents was necessary because there was insufficient signal from ^{15}N at natural abundance in PyOM samples exposed to unlabeled NH_3 or NH_4^+ . Substantial differences were observed in the ^{15}N -NMR CPMAS spectra collected from PyOM exposed to enriched ^{15}N - NH_3 gas and these differences confirmed the formation of a variety of new N functional groups, including NH_4^+ and amines ($\sim 20\text{ ppm}$), and C-N groups such as amides ($\sim 107\text{ ppm}$) and N heterocycles ($\sim 156\text{ ppm}$)^{16,33,39,40}. Similar to the results of our NEXAFS spectral deconvolution analyses, integration of the ^{15}N -NMR SEDPMAS spectrum collected from oxidized PyOM following exposure to ^{15}N - NH_3 gas shows that over 40% of the newly-incorporated NMR-detectable N is consistent with covalent C-N bonds, including more than 11% in heterocyclic structures (Supplementary Table 4). On the other hand, the ^{15}N -NMR spectrum collected from PyOM exposed to ^{15}N - NH_4^+ did not show any evidence of NH_4^+ -N incorporation into PyOM, also corresponding with the results of our NEXAFS spectral deconvolution analysis, which show very little difference between the N functional group composition of PyOM and PyOM following exposure to NH_4^+ . It is possible that some NH_4^+ -N was incorporated into PyOM, but that the quantity retained was below the detection limit for NMR, implying that it is essential for an acid-base reaction (e.g., $-\text{CO}_2\text{H} + \text{NH}_3 \rightarrow -\text{CO}_2-\text{NH}_4^+$) to occur for NH_4^+ retention.

Direct comparison of NEXAFS and NMR results is difficult because of fundamental differences between the two methods. In particular, NMR detects functional groups, while NEXAFS detects individual bonds, some of which may be present together in one functional group. Additionally, due to severe overlap of features in the $1s \rightarrow \sigma^*$ region of NEXAFS N spectra, the portion of N-H and C-NH₂ bonds present in a sample cannot be determined with this method. However, the overall results from the two analyses are consistent. In combination with stoichiometric analyses, the NEXAFS, NMR, and FTIR spectra show that PyOM interactions with NH_3 under ambient conditions can result in substantial N retention and are fundamentally different from interactions with NH_4^+ . The mechanism may involve nucleophilic NH_3 reacting with predisposed functional groups of PyOM, such as acid anhydrides, or diketo-fragments to form a range of

covalent C-N bonds, including amides and N heterocycles (Fig. 3). Similar reactions have been described between $\text{NH}_3/\text{NH}_4^+$ and small organic molecules such as carbonyls, glyoxals, and secondary organic aerosols found in the atmosphere^{23,24,41}. Both the NH_3 retention capacity and mechanisms of natural PyOM are similar to those of some industrially produced graphene oxides and activated carbons^{8,19,42} even when exposure occurs at ambient temperature and pressure. These results demonstrate for the first time that the enrichment of PyOM with N functional groups such as N heterocycles, aromatic N heterocycles, and amides may occur under natural environmental conditions and that PyOM's interaction with NH_3 versus NH_4^+ has very different implications for the global N cycle.

Discussion

Our data show that natural PyOM—a ubiquitous component of soil, atmosphere, and water—can react with NH_3 gas to form covalent bonds under conditions approximating the natural environment. This is decisive because such covalent bond formation would result in more persistent N retention than physisorption, electrostatic interactions, and precipitation of NH_4^+ salts, which are currently thought to be the dominant mechanisms for PyOM NH_3 retention⁷⁻⁹. Since covalently bound N might be less accessible to living organisms and less susceptible to volatilization, diffusion, and leaching than weakly sorbed NH_3 and NH_4^+ , it would also have very different implications for local N availability and global N cycling. By incorporating NH_3 -N into covalent C-N bonds, PyOM could provide a more dynamic mechanism for N storage, transport, and release. The discovery of aromatic and non-aromatic heterocyclic N bond formation between natural PyOM and NH_3 at ambient temperatures is also noteworthy, as this has not been observed for terrestrial pyrogenic C material, including coal, activated carbon, and graphene oxides. This is particularly relevant for industrial applications, where N-doping is used to improve the performance of C-based supercapacitors, catalysts, and other materials^{15,16,25}.

The formation of covalent bonds between PyOM and NH_3 -N under ambient conditions is a surprising outcome that has not been considered by most scientists investigating PyOM interactions with N. While the work presented here did not determine the chemical reactions responsible for such bond formation, similar reactions are well documented. For example, the reaction between carboxylic acids and amines (including NH_3 as the simplest case) to form amides is the basis of protein synthesis from amino acids. It is also well established that subsequent condensation, cyclization, and aromatization to form N-aromatics are also possible. For example, the Paal-Knorr pyrrole synthesis reaction—which produces pyrroles through the condensation of a dicarbonyl compound with an amine or NH_3 —is thought to be responsible for N heterocycle formation between secondary organic aerosols and NH_3 or amines in the atmosphere^{23,24,41}. The discovery of covalent bond formation between PyOM and NH_3 under ambient conditions may direct us to rethink PyOM material science and N biogeochemistry on local and global scales. Since many forms of organic matter present in the Earth's soil, atmosphere, and water contain the same functional groups found in PyOM, it is possible that similar reactions occur between these materials and NH_3 . Future research should investigate the extent to which organic matter retains NH_3 -N through covalent bonds, the mechanisms responsible, and the implications for global N biogeochemistry.

Given the existing uncertainties in global PyOM and NH_3 budgets, it is difficult to calculate exactly how much N might potentially be retained or transported by the Earth's PyOM stocks. Based on estimates of 54–109 Pg PyOM-C in soil¹ and an

NH₃ adsorption capacity of 13.5 mmol g⁻¹ for oxidized PyOM (Fig. 1a), we calculate that soil PyOM stocks have the potential to store or transport up to 7.3–14.7 × 10¹⁴ mol NH₃ through PyOM–NH₃ interactions, equaling up to 645-fold more than estimated annual NH₃ emissions from global agriculture, or up to 251-fold more than the estimated quantity of annually applied synthetic N fertilizer⁴³. If NH₃ interactions with soil PyOM are representative of those with other PyOM stocks, the atmospheric, ocean sediment, and marine PyOM pools could store or transport an additional 214 × 10¹⁴ mol NH₃–N through similar mechanisms. Combined, all of these PyOM stocks could retain ~320 Pg N, or more than 1500-fold the contribution of global anthropogenic N inputs per year⁴⁴.

These calculations predict a large potential influence of PyOM on global N cycling, and should motivate further work to constrain estimates so that they reflect the amount of NH₃–N retained and transported by PyOM. It is important to consider factors influencing NH₃ volatilization (e.g., pH, moisture, and temperature), PyOM–NH₃ retention capacity (e.g., functional group composition, surface area, and fouling of PyOM surfaces), and other variables that affect interactions between PyOM and NH₃ (e.g., the temperature of exposure, distance from the NH₃ source, and biological competition for NH₃). However, even at relatively low NH₃ concentrations or PyOM–N retention levels, PyOM could influence NH₃ loss, N availability to plants and microbes, and global N transport. Additional experiments are necessary to investigate the frequency of PyOM–NH₃ interactions and to examine them in more complex and heterogeneous environments, especially in marine waters and sediments, which hold the vast majority of the Earth's PyOM stocks. The coupling of global C and N cycles through such interactions could also be significant and warrants further research, particularly as global fire patterns change.

Methods

PyOM preparation. Maple (*Acer rubrum*) wood chips were pyrolyzed at 500 °C for 30 min in a modified muffle furnace²⁸. In order to produce a homogenous product, the furnace employs a custom-made inline mixing unit, regulates temperature, and maintains an internal atmosphere of inert gas throughout pyrolysis. These highly standardized process conditions ensure that the pyrolysis products are as homogenous as possible. The resulting PyOM was ground and sieved to 149–850 μm, divided into subsamples, and incubated with hydrogen peroxide (H₂O₂) or deionized water (DIH₂O) at 30 °C for up to three months (PyOM:H₂O₂ ratio of 1:10 g mL⁻¹). After oxidation, PyOM was rinsed thoroughly with DIH₂O and dried. Some PyOM samples were rewetted with DIH₂O (PyOM: DIH₂O ratio of 1:20 g mL⁻¹) and treated with 1 M HCl or NaOH until the desired pH was achieved. These PyOM samples were also rinsed with DIH₂O and dried.

PyOM characterization. PyOM pH was measured in DIH₂O at a ratio of 1:20 g mL⁻¹. SSA was quantified using the B.E.T. method with CO₂ at 273.15 K (ASAP 2020, Micromeritics, Atlanta, Georgia). Total C, N, H, and O were measured using a Delta V Isotope Ratio Mass Spectrometer (Thermo Scientific, Germany) coupled to a Carlo Erba NC2500 Elemental Analyzer (Italy).

NH₃ and NH₄⁺ adsorption. NH₃ adsorption isotherms were measured with an Autosorb iQ gas sorption analyzer (Quantachrome Instruments, Boynton Beach, Florida). Briefly, samples were degassed at 300 °C for 3 h prior to NH₃ adsorption isotherm determination, which was conducted from 80 to 800 Torr at 35 °C. Chemisorption values indicate NH₃ that was retained by PyOM under vacuum. See Supplementary Fig. 6 for comparison of N measured by chemisorption isotherms to N measured by dry combustion. For NH₄⁺ adsorption measurements, PyOM samples were mixed with 100 mM ammonium chloride solution for 16 h, filtered, rinsed with ethanol, and dried. Retained N was measured using an elemental analyzer, as described above.

FTIR. FTIR spectroscopy was used to characterize oxidized PyOM samples and investigate changes in PyOM functional group composition after exposure to NH₃ and NH₄⁺. Two replicates of each PyOM sample were scanned 200 times from 575 to 3500 cm⁻¹ at a resolution of 8 cm⁻¹ using a Bruker Hyperion FT-IR Spectrometer (Bruker, Billerica, Massachusetts) equipped with a ZnSe crystal source (PIKE Technologies, Inc., Madison, Wisconsin). Atmospheric background spectra were

subtracted from each sample spectrum. Replicate sample spectra were averaged, baseline corrected, and normalized. Wavenumbers were assigned and peak ratios were calculated for the following functional groups: 752–761, 813–823, and 875–915 cm⁻¹ to aromatic C–H out of plane deformation, 1690–1715 cm⁻¹ to carbonyl/carboxyl and ketonic C=O stretching, and 1581–1609 cm⁻¹ to aromatic C=C vibrations and stretching (OPUS, Bruker, Billerica, Massachusetts).

NEXAFS. Nitrogen K-edge NEXAFS was used to discern how NH₃ and NH₄⁺ were retained by PyOM following exposure. Briefly, samples were mounted onto gold-coated silicon wafers and scanned in 49 different locations for 20 seconds each, without any spatial overlap to prevent radiation damage to the sample. N K_α partial fluorescence yield was collected using silicon drift detectors in the slow scanning mode of the spherical grating monochromator beamline at the Canadian Light Source (Saskatoon, Canada). For each sample, all 49 scans were averaged across four detectors and normalized by the beamline incident flux obtained by measuring the drain current in a gold mesh (IGOR Pro 6.36, WaveMetrics, Lake Oswego, Oregon). Following a modification of the method used by Gillespie et al.⁴⁵, spectra were shifted based on the N₂ absorption spectrum measured from ammonium sulfate, background corrected, smoothed, and normalized to an edge-step of 1 (Athena 0.8.056, Bruce Ravel; Ifeffit 1.2.11, Matt Newville, University of Chicago, Chicago, Illinois). Deconvolution was performed using Gaussian curves and peak characteristics of N-containing standards (Fityk 0.9.8, Marcin Wojdyr; see Supplementary Fig. 7 for N standard spectra, Supplementary Table 1 for peak assignments used in deconvolution, and Supplementary Table 3 for features in spectra collected from standard compounds). The fraction of π* area associated with specific N bonds (compared to total area of all deconvolution products) was calculated for each sample (Supplementary Table 2). If they were present as physically adsorbed molecules, neither NH₃ nor NH₄⁺ could have been responsible for the development of the numerous pre-edge features in the spectra collected from PyOM that was exposed to NH₃ and NH₄⁺ (see Supplementary Figs. 7 and 8, Supplementary Table 2, and refs. 34–38).

To confirm that radiation damage was not responsible for spectral features, samples were also scanned 15 additional times in the same location. These 15 spectra were then averaged, shifted, background corrected, smoothed, and normalized as described above. If the samples were susceptible to beam damage, we would expect to see new spectral features that would become more pronounced as each additional scan exposed the sample to increasing radiation. However, as shown in Supplementary Fig. 9, this did not occur—even after a 15-fold increase in radiation, the spectral features remain the same as those presented in Fig. 3a. As other authors have noted, this indicates that radiation damage was not responsible for the features present in NEXAFS sample spectra⁴⁶.

Solid-state NMR spectroscopy. Solid-state NMR spectroscopy was used to investigate how NH₃ and NH₄⁺ were retained by oxidized PyOM following exposure. In order to obtain a sufficiently strong signal during ¹⁵N-NMR experiments, oxidized PyOM samples were exposed to gaseous [¹⁵N]-NH₃ with 98 atom% ¹⁵N (Air Liquide America Specialty Gases, Plumsteadville, PA) and [¹⁵N]-NH₄⁺ with 10 atom% ¹⁵N (Cambridge Isotope Labs, Tewksbury, MA) at 35 °C, under atmospheric pressure.

¹D ¹H, ¹³C and ¹⁵N solid-state NMR spectra were obtained at a magnetic field of 7 Tesla (¹H, ¹³C and ¹⁵N Larmor frequency of 300 MHz, 75 and 30 MHz, respectively) using a Bruker Avance III NMR spectrometer fitted with a 4 mm magic angle spinning (MAS) double resonance probe. For both the ¹³C and ¹⁵N experiments, ~50 mg of PyOM was packed into a 4 mm zirconia rotor sealed with a Kel-F cap. For ¹³C experiments, CP (cross-polarization) was achieved with 6.5 kHz MAS; contact time, 1 ms, ramped from 70 to 100%; recycle delay, 3–20 s (Supplementary Figs. 10 and 11); 83 kHz ¹H decoupling via spinal-64 sequence; ca 2k scans; TOSS (TOtal Suppression of Spinning side-bands) removed side-bands from the aromatic peaks that obscured the aliphatic region, and also gave better quality response from the sample without pulse breakthrough (Supplementary Figs. 4 and 12); SEDP (spin echo direct polarization with ¹H decoupling) was achieved with 12 kHz MAS; recycle delay of 2–150 s; ¹³C excitation pulse of 4.5 μs (90°); echo time of ~75 μs; 71 kHz ¹H decoupling via spinal-64 sequence (Supplementary Fig. 13). Complementary spectra were also acquired with dipolar-dephasing delays of 40 μs with both CP and SEDP to assess non-protonated carbon content. For ¹⁵N experiments, CP was achieved at 5 kHz MAS; contact time, 2 ms, ramped from 70 to 100%; recycle delay, 3 s; 83 kHz ¹H decoupling via spinal-64 sequence⁴⁷. TOSS was also used to suppress pulse breakthrough. For ¹⁵N SEDPMAS experiments, 10 kHz MAS was used with 100 μs echo time and a 50 kHz ¹H decoupling field during acquisition with the spinal-64 sequence. The relaxation behavior was tested using a series of experiments with a fixed number of scans (400) and with increasing recycle delays from 5 to 400 s (Supplementary Figs. 14 and 15).

All spectra were processed using the Bruker software, TOPSPIN 3.5pl7. Spectra were produced from the free induction decays by first zero filling, applying Gaussian multiplication (e.g., LB = -10, GB = 0.03), Fourier transformation, and phase correction. Chemical shift values were referenced to the C=O of glycine, δ_C 176 ppm for ¹³C and to (NH₄)₂SO₄, δ_N 24 ppm on the NH₃ scale for ¹⁵N. All literature quoted in the text were converted to the NH₃ scale for ¹⁵N by adding 380 ppm.

Data analysis. All statistical analyses were performed using the lsmmeans and nlstools packages^{48,49} in the statistical computing language and environment R⁵⁰.

Data availability

The data that support the findings of this study are available in Cornell University's digital repository eCommons with the identifier <https://doi.org/10.7298/X0B7-PX55>.

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Author contributions

R.H. and J.L. conceived the experiments; R.H. performed the experiments and analyzed the data; D.T.-R., J.D., A.G. and T.R. assisted with NEXAFS measurements and interpretation; J.H. conducted the NMR experiments; J.H. and R.S. led NMR data interpretation; R.H. wrote the paper; all authors contributed to the final draft.

Additional information

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