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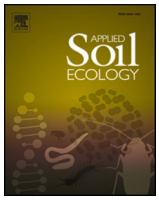

Research paper

Cicada necrobiome mediates greenhouse and trace gas pulses following periodic mass emergence

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ABSTRACT

The emergence of periodical cicadas from soil every 13 or 17 years is a unique ecological phenomenon with the potential to affect soil biogeochemistry in forests, with increased emissions of climate-relevant gases as a consequence. While it's well-known that cicada carcasses create resource pulses of carbon and nitrogen (N) in soil when they die in mass, the processes underlying these effects, as well as the consequences of these effects for N losses, are poorly known. We investigated how the emergence of Brood X cicadas (*Magicicada* spp.) in 2021 affected soil microbial communities – particularly N cycling taxa - in forests of the United States. We found that decaying carcasses led to emissions of nitrous oxide (N_2O) and ammonia (NH_3) gas at around 0.53 mg-N m^{-2} h^{-1}, estimated to be a ~ 35-fold increase over ~21 days from the annual average emissions from US forest soils (0.015 mg-N m^{-2} h^{-1}), with the greatest effects occurring at the interface between carcasses and soil surface. Using amplicon sequencing and qPCR, we determined the potential microbial mechanisms behind N_2O and NH_3 production, including correlations between taxa capable of carrying out less well studied processes DNRA and nitrifier denitrification, and increased emissions of N_2O and NH_3. Although distinguishing the relative contributions of DNRA, denitrification, and nitrifier denitrification requires direct rate measurements, our results suggest these processes working together contribute to previously unrecognised greenhouse gas emissions following insect emergence events. Collectively, our results indicate that cicadas significantly affect nutrient cycling in forests with the potential to alter soil microbial communities in ways that may enhance ecosystem N emissions.

1. Introduction

Periodical cicadas (*Magicicada* spp.) are a unique group of insects with a distinctive life cycle, which includes long periods of development underground (13 or 17 years) followed by synchronised mass emergence (Lehmann-Ziebarth et al., 2005). Periodical cicadas typically occur in the eastern and midwestern United States (U.S.) and are grouped into different "broods". There are currently 12 active 17-year cicada broods and 3 active 13-year cicada broods distributed across the U.S. (Cooley et al., 2009). The synchronised emergence of periodical cicadas may have evolved to overwhelm predators, and indeed the majority of adult cicadas are not eaten, instead reaching the end of their life cycle after 6–8 weeks aboveground and falling to the soil surface (Lloyd and Dybas, 1966; Menninger et al., 2008; Williams and Simon, 1995). However, the biogeochemical consequences of these synchronous emergences remain poorly understood. The exoskeletons of cicadas and other insects are primarily made up of chitin, a nitrogen (N) rich polymer of *N*-acetylglucosamine (GlcNAc), and one of the most abundant amino-polysaccharide polymers in nature (Elieh-Ali-Komi and Hamblin, 2016). Chitin can be broken down to GlcNAc, glucosamine (GlcN), and other derivatives by bacteria and fungi that produce chitinolytic extracellular enzymes (e.g., *N*-acetylglucosaminidase or NAGase). Given the size of most cicada broods (~30,000-3,500,000 insects per hectare) and a mean mass per insect of ~200 mg (Setälä et al., 2022; Yang, 2004), inputs of biomass from cicada carcasses are on average ~ 350 kg ha^{-1}, which generates a large pulse of carbon (C) and N to soil. How such inputs affect biogeochemical cycling and fluxes of climate-relevant gases from soils is largely unknown (Williams and Simon, 1995).

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The massive potential quantity of cicada biomass deposited on soils represents a uniquely large input of N-rich organic matter, and therefore nutrients to soils, which likely stimulates biogeochemical cycling, particularly of N. Consequently, this organic matter input may be significant for global and regional emissions budgets by stimulating soil emissions of nitrous oxide (N_2O), a potent greenhouse gas, and ammonia (NH_3), which contributes to climate change (Reay et al., 2018; Wyer et al., 2022). Following cicada emergence and death, chitin-derived GlcN provides an abundant source of organic N (R-NH_2) which can be microbially mineralised to produce ammonium (NH_4^+) (Fig. 1). When NH_4^+ is present in the soil, especially in alkaline conditions, it can convert to NH_3 and escape into the atmosphere. This volatilisation is particularly common following the addition of urea-based fertilisers or organic matter. Although NH_3 itself is not a greenhouse gas, it contributes indirectly to climate forcing by reacting with sulphuric and nitric acids to form secondary inorganic aerosols, such as ammonium nitrate and ammonium sulphate. These aerosols are major components of particulate matter ($PM_{2.5}$), which influences climate by scattering and absorbing solar radiation and by acting as cloud condensation nuclei, thereby affecting cloud properties and radiative forcing (Wang et al., 2025). Oxidation of inorganic compounds NH_4^+/NH_3 by ammonia oxidising bacteria (AOB) and archaea (AOA), is the first step in the dominant process of autotrophic nitrification, however GlcN is also a potential source of organic N from the amine group ($-NH_2$) for heterotrophic nitrification (HN). In soils with higher organic matter content heterotrophic nitrifiers can oxidise a broader range of N compounds (Stein, 2014), which may increase the relevancy of this process in soils amended with cicada carcasses. Although both methods of nitrification are capable of producing N_2O as a by-product, HN produces significantly more under certain environmental conditions (Zhang et al., 2015). The subsequent increased availability of NO_3^- from nitrification has the potential to drive higher rates of denitrification than are seen in unamended soils. During denitrification, N can be emitted from the soil as NO and N_2O gas if denitrifying microbes do not have necessary enzyme-encoding genes for the subsequent steps of the denitrification process to form N_2 (Cuhel et al., 2010; Richardson et al., 2009). Some ammonia oxidising taxa, primarily AOB, also have the capability for an alternative denitrifying process called nitrifier denitrification, which may be responsible for a significant proportion of soil-sourced N_2O emissions, depending on the environmental conditions (Wrage-Mönnig et al., 2018). Nitrifier denitrification is favoured by low pH conditions, which are common in forest soils such as those studied here. High NO_2^- conditions, such as those that may result from NH_4^+ oxidation following decomposition of carcasses at the soil-carcass interface, also promote nitrifier denitrification (Wrage-Mönnig et al., 2018). Alongside denitrification, dissimilatory nitrate reduction to ammonium (DNRA) can be a source of N_2O - a by-product of the reduction of NO_3^- to NH_3 in soils (Giles et al., 2012). Whether DNRA or denitrification dominate NO_3^- reduction is dependent on many biotic and abiotic factors including redox conditions, C:N, NO_2^-:NO_3^-, and rate of bacterial growth (Pandey et al., 2020). ^{15}N tracing studies have demonstrated that forest soils could support significant DNRA activity due to high organic matter content. As an anaerobic process, DNRA may be favoured in oxygen (O_2)-limited conditions, such as microenvironments that can arise during microbial O_2 consumption as a result of organic matter decomposition, though NO_3^- concentration is a more significant predictor of DNRA rates (Rütting et al., 2011; Keiluweit et al., 2018). In forest soils amended with high organic matter content in the form of cicada carcasses, potentially leading to high NO_3^- concentration at the soil-carcass interface, understudied processes such as DNRA have the potential to be increasingly significant for soil N cycling and N_2O production. While functional gene abundance can indicate the potential for specific microbial processes, it is important to note that gene presence does not necessarily equate to in situ activity. The relative contributions of competing N reduction pathways (e.g. DNRA vs. denitrification) are challenging to quantify without isotopic tracing approaches. Nevertheless, characterising the microbial functional potential provides valuable insights into the mechanisms underlying ecosystem-scale gas fluxes.

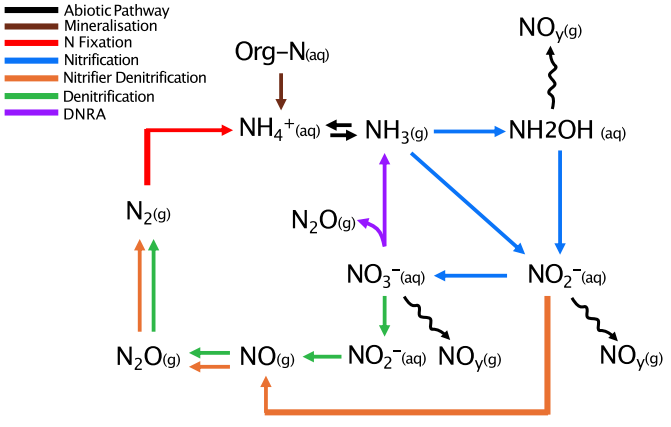

Fig. 1. Overview of nitrogen cycle processes: organic nitrogen mineralisation, nitrogen fixation, nitrification, nitrifier denitrification, denitrification, and dissimilatory nitrate reduction to ammonia.

Resource pulses owing to insects are common in forests, with the magnitude of effects depending on the frequency and duration of the disturbance (Jentsch and White, 2019). While living periodical cicadas generally have minimal impacts on soil resources (Setälä et al., 2022), their mass die off 6–8 weeks post-emergence can alter soil N cycling for weeks to months (Yang, 2004). Biotic ecosystem disturbance in the context of periodical cicada emergences demonstrates how alterations to the environment can lead to widespread consequences, including shifts in species composition with certain predators exploiting the abundant cicada resource and plants and microorganisms experiencing changes in nutrient dynamics (Maurice et al., 2024). Previous studies on outbreaks of defoliating insects in forests have quantified greater effects on intra-system transfers of N as opposed to N losses (Lovett et al., 2002). Whether the periodicity of cicadas influences their impacts on ecosystem N loss is not currently known. Further, periodical cicada emergences are an example of predictable events that influence ecosystem functioning over long timescales. In this way, these events may differ from the effects of unexpected catastrophic, large-scale biotic disturbances, such as outbreaks of other insect species, or extreme weather events (Setälä et al., 2022; Yang, 2004).

Brood X are 17-year periodical cicadas and are one of the largest broods in the eastern U.S. (Ficklin et al., 2023). We carried out our study on the Brood X emergence in the summer of 2021 in Indiana (IN) (Kritsky, 2021). In this study we investigate responses of soil biogeochemistry following periodical cicada emergence in forests. We hypothesised that forest soils act as “hotspots” of gas emissions, stimulated by enhanced microbial activity, carcass decomposition and nutrient release. In the field, we collected and added cicada carcasses to static flux chambers to analyse the effects of carcass presence on soil gas fluxes. In lab-based experiments, cicada carcasses were allowed to decompose for 21 days, while gas flux measurements, quantification of microbial community composition, and nutrient analyses were carried out throughout to examine how the microbiology and chemistry occurring at the soil surface-cicada carcass interface affects emissions of gases from forest soils over time.

2. Methods

2.1. Experimental site

The experimental plots were located in Griffy Woods in southern IN at 39.191436°N, −86.500348°W and 39.191377°N, −86.500354°W, which resides within Indiana University's Research and Teaching Preserve. Griffy Woods is a deciduous hardwood, secondary growth forest

containing a diverse mixture of tree species, including tulip poplar (*Liriodendron tulipifera*), red oak (*Quercus rubra*), white oak (*Quercus alba*), black oak (*Quercus velutina*), bitternut hickory (*Carya cordiformis*), and sugar maple (*Acer saccharum*). While vegetation structure can influence soil microclimate and microbial activity, the present study focuses on observed N_2O and NH_3 fluxes under prevailing field conditions; explicit canopy metrics (e.g., LAI or basal area) were not measured and thus potential mechanistic links remain an avenue for future research. Soil textures at the site can be found in **Table S1.** of the supporting information. Sites had a mean annual precipitation of 1200 mm and mean annual temperature of 11.6 °C (Beverly et al., 2024). Soil and air temperatures (°C), and soil moisture content (%) over the sampling period can be found in **Table S2.** of the supporting information.

2.2. In situ gas flux measurements

Brood X cicada carcasses were collected around Griffy Woods as well as other locations in Bloomington, IN, U.S. Carcasses were added to the soil surface inside static flux chambers. Two chambers had a "low" cicada treatment (~200 carcasses m^{-2}), and six chambers had "high" cicada treatment (~500 carcasses m^{-2}). These densities encompass the range reported for periodical cicada emergence holes (Beverly et al., 2024; Pray et al., 2009; Pruitt et al., 2026) and are therefore biologically plausible for carcass densities given the synchronous and spatially heterogeneous nature of *Magicicada* populations. Two control chambers contained no cicada carcasses, giving a total of 10 chambers. Chambers were open to air but covered with netting between measurements to prevent carcasses from being consumed by birds, mammals, and insects. To quantify N_2O emissions from static flux chambers, 0.56 L air was sampled from each chamber four times at 20 min intervals and collected in Tedlar bags. Measurements were conducted four times at 2, 4, 10, and 18 days post cicada carcass addition in August 2021. Sample analysis was performed in the lab using an Aerodyne TILDAS N_2O Analyser for N_2O quantification. Flux calculations are detailed in **Method S1**. of the supporting information.

2.3. Lab based gas flux measurements

Six chambers were set up in the laboratory as in **Fig. S1**. 200 g of sieved soil collected from Griffy Woods was added to each chamber and normalised to 20–30% moisture content with DI water. Two cicada carcasses were added to four microcosms, giving a density approximately equivalent to 250 cicadas m^2, ensuring no overlap between carcasses. This density falls within the range investigated during the field experiments. One microcosm served as a blank with only soil, and one microcosm served as a control with no soil or cicada carcasses. Fluxes of N_2O and NH_3 were measured with continuous gas sampling over 20 days using an atmosFIR FTIR spectrometer (Protea, U.K.). The blank was used to mitigate any soil effects and the control was used to mitigate any microcosm effects. Flux calculations are detailed in **Method S1**. of the supporting information. Microcosms were connected to a multiplexer such that data was collected for 6 mins per hour for each chamber. Concurrently, 5 cm diameter petri dishes were set up with 10 g of soil and one cicada carcass each, and incubated at 25°C. Every 3 days for 21 days, petri dishes were destructively sampled and four replicates of 5 g of soil were collected, giving a total of 28 soil samples. The cicada carcasses were collected and washed as in **2.4**.

2.4. Characterisation of soil microbiome and cicada necrobiome

Cicada carcasses were washed to collect the surface microbiome, termed here the "necrobiome". Carcasses were placed in tubes containing 20 mL 0.1% Tween 20 and sonicated for 15 mins, vortexing every 5 mins for 10 s. Tubes were centrifuged for 10 mins at 3000 rpm and the pellet was resuspended in 1 mL DI H_2O. Tubes were then centrifuge for 2 mins at 15,000 x *g* and the pellet was resuspended in 100 μL DI H_2O, forming what is termed here as "cicada washes". Soils and cicada washes were stored at −80 °C immediately after collection and prior to analysis. The Qiagen DNeasy PowerSoil Kit protocol was used to extract DNA from 0.25 g subsamples of soil, or 100 μL of cicada washes. Concentrations of eluted DNA were quantified using a Qubit assay according to the manufacturers protocol. Primers 515f (5'-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5'-GGACTACHVGGGTWTCTAAT-3′) were used to amplify 16S rRNA gene fragments from the extracted DNA. Illumina MiSeq Nano was used for 250 bp, paired-end sequencing of amplicons. Qiime2 (v 2022.2.0) was used to analyse fastq files from 16S rRNA amplicon sequencing (Bolyen et al., 2019). Primers were trimmed from sequences, and sequences were demultiplexed using the cutadapt tool. DADA2 was used to denoise sequences and merge paired ends. Taxonomy was assigned to operational taxonomic units (OTUs) using the Greengenes full length 16S rRNA gene database (McDonald et al., 2012). Mitochondria and chloroplasts were removed. OTUs were assigned to functional groups using Functional Annotation of Prokaryotic Taxa (FAPROTAX)(v 1.2.6). V 1.3.5 of the collapse_table.py script was used with python3 (v 3.8.9 64-bit) (Van Rossum and Drake, 1995). Quantitative PCR (qPCR) was carried out on an Agilent Technologies Stratagene Mx2005p qPCR system with FastStart SYBR Green Master (Roche Diagnostics GmbH, Germany) with FastStart Taq DNA Polymerase to quantify the activity of bacterial and archaeal amoA, and fungal p450nor (98 °C, 3 mins; 40 cycles of 98 °C, 15 s; 60 °C, 30 s). Details of qPCR conditions and calculations of gene abundance can be found in **Table S3**. and **Method S2**. of the supporting information.

2.5. Respiration

Respiration rate ($mgCO_2$-produced $kg\text{-}soil^{-1}$ day^{-1}) was measured using OxiTop®-IDS Multi 3630/3620 respirometers (WTW Xylem Analytics, Germany) according to DIN ISO 16072 from bottles containing 150 g soil from Griffy Woods with and without the addition of cicada carcasses. Bottles were incubated at 20 °C and measurements were collected each day for 7 days. Respiration rate was calculated as in **Method S3** of the supporting information.

2.6. Quantification of carbon and nitrogen concentrations

Carbon and nitrogen content of soil subsamples and cicada carcasses was quantified using a Costech Elemental Analyser ECS4010 (Costech Analytical Technologies, CA, USA). Sample preparation was as follows: Soil samples and cicada carcasses were dried at 60 °C for 48 h. For each sample, 1 g soil was weighed into a 2 mL stainless steel grinding tube with two stainless steel 5 mm ball bearings. Tubes were sealed with silicon bungs. A FastPrep-24™ Classic bead beating grinder (MP Biomedicals, CA, USA) was used to grind the samples in 1 min increments 12 times.

2.7. Quantification of nitrate and ammonium concentrations

Soil samples were shaken in 0.01 M $CaCl_2$ for 1 h at 180 rpm to produce extractions of the inorganic N (nitrate and ammonium) present. Nitrate and ammonium concentrations of soil extractions and cicada washes were quantified using a FLUOstar® Omega filter-based multimode microplate reader. 0.01 M $CaCl_2$ was used for the extractions as the Ca^{2+} ions in the solution can effectively displace NO_3^- and NH_4^+ ions from soil particles into the extraction solution. Absorbances used were 540 nm for NO_3^- and 667 nm for NH_4^+. Concentrations of standards were 8.0, 4.0, 2.0, 1.0, 0.5, and 0.25 mg L^{-1}. Details of chemistry can be found in **Method S4** of the supporting information.

2.8. Statistical analyses

Measurements of gas fluxes in the field were made from 10

chambers. Measurements of potential gas fluxes in the lab were made from 6 microcosms, with 2880 datapoints per sample. Analysis of microbial communities from field chambers was carried out on 10 samples at 0–5 cm and 5–15 cm depth. Analysis of microbial communities from the time series experiments was carried out on 64 samples. Measurements of respiration rates were made from 6 OxiTop® respirometers, with one datapoint per day. Quantification of NO_3^- and NH_4^+ concentrations was carried out on 32 samples. Quantification of C:N was carried out on 15 cicada carcass samples. Statistical tests were performed using R (v 4.1.2). Dataset normality was tested with Shapiro-Wilk tests. As the dataset was concluded to be non-normally distributed, Kruskal-Wallis rank sum tests were used (KW) to assess the relationship between measured variables and cicada treatment, with *p*-values corrected for multiple comparisons with Dunn's tests using the false discovery rate with the Benjamini-Hochberg methods using R package FSA (v 0.9.4). To assess relationships between measured variables, Spearman's rank correlation coefficient tests (SRCC) were used. Significant differences were inferred when $p < 0.05$.

3. Results

3.1. Quantification of gas fluxes

From the initial field experiments, mean fluxes of N_2O (F_{N2O}) from chambers amended with cicada carcasses (28,849 ± 13,655 ng-N m^{-2} h^{-1}) were significantly higher than from control chambers (2472 ± 459 ng-N m^{-2} h^{-1}) (KW; $p < 0.01$), and peaked at day 10 after addition (Fig. 2A). Flux data from chambers with high and low density cicada additions was pooled to increase the sample size for statistical analysis as there was no significant difference in measured fluxes between these treatments. From the time series experiment, mean potential fluxes of NH_3 (F_{NH3}) and F_{N2O} were significantly positively correlated. F_{N2O} and F_{NH3} were significantly higher from chambers amended with cicada carcasses (2110 ± 58 and 2147 ± 130 ng-N m^{-2} h^{-1}, respectively) than control chambers (−112 ± 8 and − 2396 ± 162 ng-N m^{-2} h^{-1}, respectively) (KW; $p < 0.001$, for both), and significantly increased with time (KW; $p < 0.001$, for both). F_{N2O} and F_{NH3} peaked at day 15 of the time series experiment with a value of 4300 ± 474 and 2392 ± 169 ng-

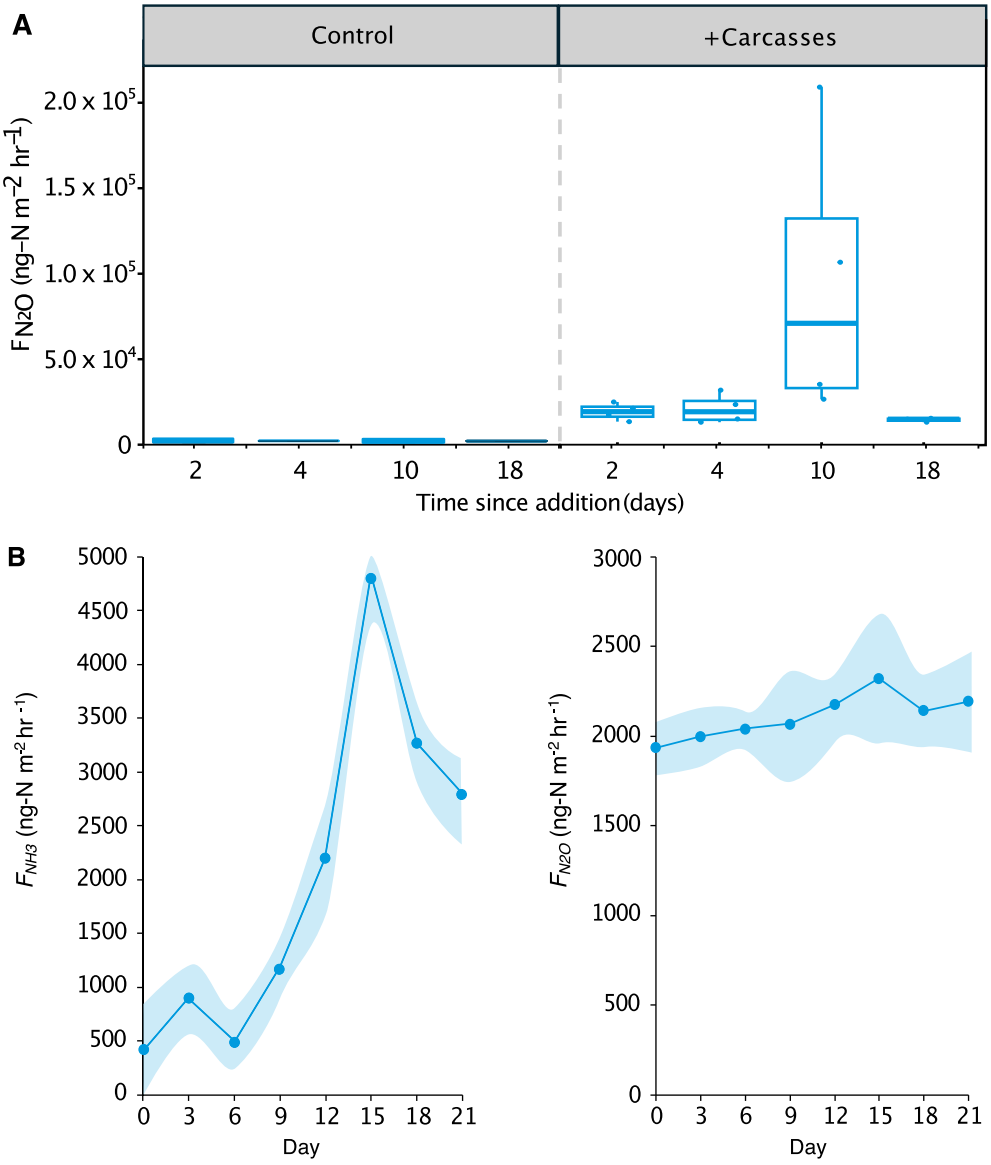

Fig. 2. In situ fluxes of N_2O (F_{N2O}) from Indiana forest soils with and without cicada carcass amendment, measured using an Aerodyne TILDAS N_2O Analyser on samples collected from static flux chambers. $N = 10$. **B.** Fluxes of NH_3 (F_{NH3}) and F_{N2O} from lab-based microcosms containing soils with cicada carcass amendment, measured over 21 days. Gases were quantified using a continuous flow atmosFIR FTIR instrument. Shading indicates standard error. $N = 6$.

N m^{-2} h^{-1}, respectively (Fig. 2B).

3.2. Characterisation of soil microbiome and cicada necrobiome

From 16S rRNA amplicon sequencing of soil samples from control field chambers and those amended with cicada carcases, a total of 246,018 reads were obtained from 20 samples. The average reads per sample was 15,012. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) showed that microbial community compositions of samples of soil were not significantly different between control chambers and those amended with cicada carcasses. From 16S rRNA amplicon sequencing of soil and cicada wash samples from the lab-based decomposition study, a total of 1,078,950 reads were obtained from 64 samples. The average reads per sample was 15,866. The composition of the soil microbiome and the cicada necrobiome was significantly different (ANOSIM; $p < 0.05$). The dominant N cycle taxa in the soil microbiome were *Rhizobiales* (24.77% of the N cycle community) and the dominant N cycle taxa in the cicada necrobiome were *Bacillales* (24.45% of the N cycle community). Abundances of key microbial taxa relative to the total community and their potential functions for the soil microbiome and cicada necrobiome can be found in **Table S4.** and **Table S5.** of the supporting information, respectively.

Following functional annotation of OTUs using the FAPROTAX database, there were significantly higher counts of taxa, with the functional capacity for chitinolysis and DNRA in cicada necrobiome samples compared to soil microbiome samples (KW; $p < 0.05$). *Enterobacterales* (predominantly *Serratia marcescens*) are capable of both chitinolysis and DNRA, and represented 9.6% of the total necrobiome community and 4.3% of the total soil microbiome community at the peak of F_{N2O} and F_{NH3}. Abundance of chitinolysis taxa peaked on day 0 and abundance of DNRA taxa peaked on day 15. (Fig. 3.). Additionally, there were significantly higher counts of microbes with the functional capacity for N fixation, ammonia oxidation, nitrification, and denitrification in soil microbiome compared to cicada necrobiome samples (KW; $p < 0.001$, for all.) In cicada necrobiome samples, taxa with the functional potential for DNRA and denitrification were significantly correlated with taxa with the functional potential for chitinolysis and ureolysis (SRCC; $p < 0.01$, for both). Taxa with the functional potential for N fixation were significantly correlated with taxa with the functional potential for ammonia oxidation, nitrification, and denitrification (SRCC; $p < 0.01$, for all). F_{N2O} from chambers amended with cicada carcasses were significantly positively correlated with microbes with the functional potential for DNRA and denitrification in the cicada necrobiome (SRCC; $p < 0.05$). We note that while these correlations between DNRA taxa abundance and N_2O emissions suggest microbial activity, they do not quantitatively partition N_2O production among DNRA, conventional denitrification, and nitrifier denitrification pathways.

qPCR standard curves had $R^2 > 0.95$ and efficiency values between 90 and 100%. There were significantly higher copies g-$soil^{-1}$ of bacteria

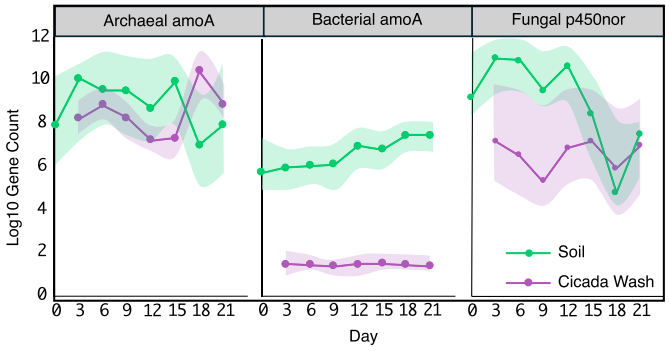

Fig. 4. Log10 gene counts of nitrogen cycle genes archaeal and bacterial ammonia monooxygenase (amoA), and fungal nitrous oxide reductase (p450nor) from soil and cicada washes, collected every 3 days for 21 days. Gene counts were quantified using quantitative PCR. Shading indicates standard error. $N = 64$.

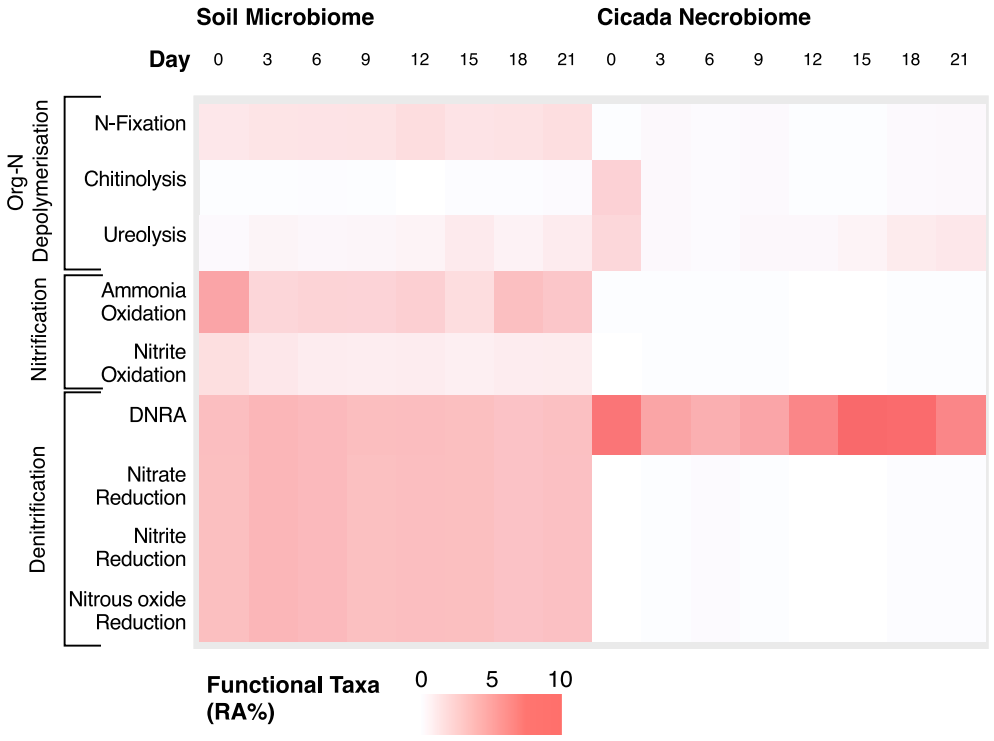

Fig. 3. Heat map of relative abundances of nitrogen cycle specific functional taxa in the soil microbiome and cicada surface microbiome (the "necrobiome"). Microbial communities were quantified with 16S rRNA amplicon sequencing on Illumina MiSeq Nano. Colour scale is consistent across relative abundances of both the soil microbiome and cicada necrobiome functional taxa. $N = 64$.

amoA in soil samples compared to cicada wash samples (Fig. 4). Copies g-soil^{-1} of bacterial amoA were significantly positively correlated with microbes with the functional capacity for chitinolysis and ureolysis in soils (SRCC; $p < 0.01$, $p < 0.05$, respectively), with cicada wash NO_3^- concentration (SRCC; $p < 0.05$), and with F_{N2O} and F_{NH3} from cicada-amended microcosms (SRCC; $p < 0.001$, for both). Copies g-soil^{-1} of bacterial amoA were significantly negatively correlated with copies g-soil^{-1} of archaeal amoA (SRCC; $p < 0.05$) and p450nor genes (SRCC; $p < 0.01$), and with soil NO_3^- concentration (SRCC; $p < 0.001$) and soil NH_4^+ concentration (SRCC; $p < 0.05$). Copies g-cicada^{-1} of bacterial amoA were significantly positively associated with copies g-cicada^{-1} of p450nor (SRCC; $p < 0.05$). Copies g-cicada^{-1} of archaeal amoA were significantly positively associated with microbes with the functional capacity for nitrification in cicada washes (SRCC; $p < 0.05$). Copies g-cicada^{-1} of archaeal amoA were significantly negatively associated with cicada wash NH_4^+ concentration, and abundance of microbes with the functional capacity for ureolysis (SRCC; $p < 0.05$, for both). Copies g-soil^{-1} of p450nor were significantly positively associated with copies g-cicada^{-1} p450nor (SRCC; $p < 0.05$), and significantly negatively associated with F_{NH3} from cicada-amended microcosms (SRCC; $p < 0.05$).

3.3. Respiration rate and C:N

Respiration rate from soil samples (mg-CO_2 produced kg-soil^{-1} d^{-1}) and from cicada samples (mg-CO_2 produced kg-cicada^{-1} d^{-1}) both increased significantly over decomposition time (SRCC; $p < 0.001$) (Fig. 5). The mean respiration rate was significantly higher with cicada treatment (KW; $p < 0.01$). C:N of cicada carcasses was significantly higher after decomposition (day 21) compared to before decomposition (day 0) (KW; $p < 0.05$).

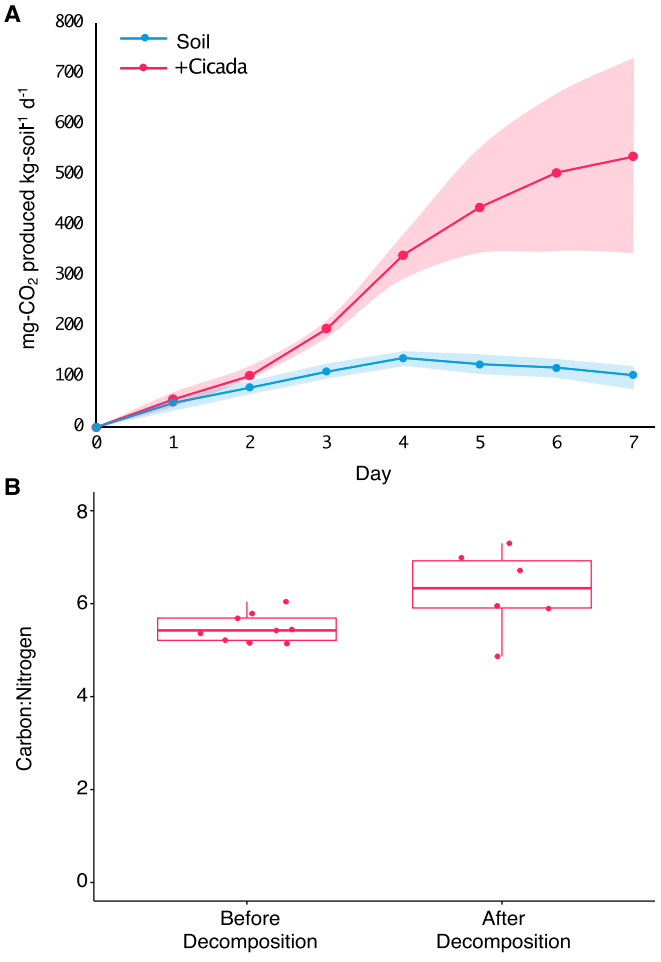

Fig. 5. Respiration rate (mg-CO_2-produced kg-soil^{-1} d^{-1}) quantified from soil with and without cicada amendment using OxiTop®-IDS Multi 3630/3620 respirometers measured over 7 days. Shading indicates standard error. $N = 6$. **B.** Carbon:nitrogen of cicada carcasses before and after decomposition for 21 days at 25°C measured with a Costech Elemental Analyser ECS4010. $N = 15$.

3.4. Quantification of nitrate and ammonium concentrations

Average NO_3^- concentration was significantly higher in soil samples than cicada samples (KW; $p < 0.001$) (Fig. 6A). However, soil NO_3^- was significantly positively correlated with the abundance of nitrification taxa in cicada wash samples (SRCC; $p < 0.05$). Soil NO_3^- concentration was also significantly positively correlated with decomposition time (SRCC; $p < 0.001$) (Fig. 6B). Average soil NO_3^- concentration was significantly positively correlated with abundance of chitinolysis taxa in soil samples (SRCC; $p < 0.01$). Average soil NO_3^- concentration was also significantly positively correlated with F_{N2O} and F_{NH3} from cicada-amended microcosms (SRCC; $p < 0.01$, $p < 0.001$, respectively) (**Fig. S2** of the supporting information). Average soil NO_3^- concentration was significantly negatively associated with average soil NH_4^+ concentration (SRCC; $p < 0.05$). Average cicada wash NO_3^- concentration was significantly positively associated with F_{N2O} from cicada-amended microcosms (SRCC; $p < 0.05$).

4. Discussion

Periodical cicada emergence represents a large and predictable ecosystem disturbance, with understudied consequences for soil biogeochemistry and subsequent emissions of climate-relevant gases. We hypothesised that U.S. forest soils may act as "hotspots" of gas emissions through stimulation of microbial activity by nutrient release from cicada carcass decomposition. Our emissions data shows that the annual mean of 129.67 mg-N m^{-2} lost as N_2O and NH_3 combined from US forest soils could theoretically be lost in just ~10 days at the rate of 0.53 mg-N m^{-2} h^{-1} that we estimate in the presence of decaying cicada carcasses, with significant implications for regional reactive N budgets.

The increase in N_2O and NH_3 loss from soils is due to stimulation of soil N cycling processes fuelled by the large input of organic matter as cicada carcasses. In particular, ammonia oxidation is potentially a dominant process at the onset of carcass decomposition as the highest abundance of taxa with the potential to carry out this process in the soil, and highest abundance of the AOA gene in the soil were quantified by amplicon sequencing and qPCR in the early stages of the time series experiment (day 0 to 6). This is also consistent with a concurrently high concentration of available NH_4^+ at this time. Denitrification then becomes more dominant as soil NO_3^- concentration increases and is available for reduction to NO, N_2O and N_2. Interestingly, our results indicated that the most abundant chitinolytic microbes may also be able to opportunistically utilise available NO_3^- for DNRA, a competing process with conventional denitrification, that potentially contributes to the pulses of N_2O and NH_3 gases.

However, the pKa of NH_4^+ is approximately 9.25, meaning that at a lower pH, the equilibrium favours this form. Therefore, DNRA does not emit NH_3 directly unless the soil has a pH high enough to permit its release via volatilisation. Most forest soils are neutral to slightly acidic, between pH 5.0–7.0, including those in this study (ranging from pH 6.2 to pH 6.6), which would not favour the volatilisation of NH_3. Insect haemolymph is generally neutral to alkaline, varying between pH 6.4–8.0 (Glaser, 1925), contributing to a rise in soil pH as the insect decomposes that could lead to NH_3 volitalisation and emission. Even if bulk soil pH is not particularly high, there can be significant NH_3 emissions due to the formation of alkaline microsites during decomposition, where NH_3 is formed and volitalises before it diffuses to more acidic soil (Yan et al., 1996). DNRA will also contribute to increasing NH_4^+ concentration within the soil which can then be nitrified and denitrified to form N_2O. The formation of alkaline microsites could also

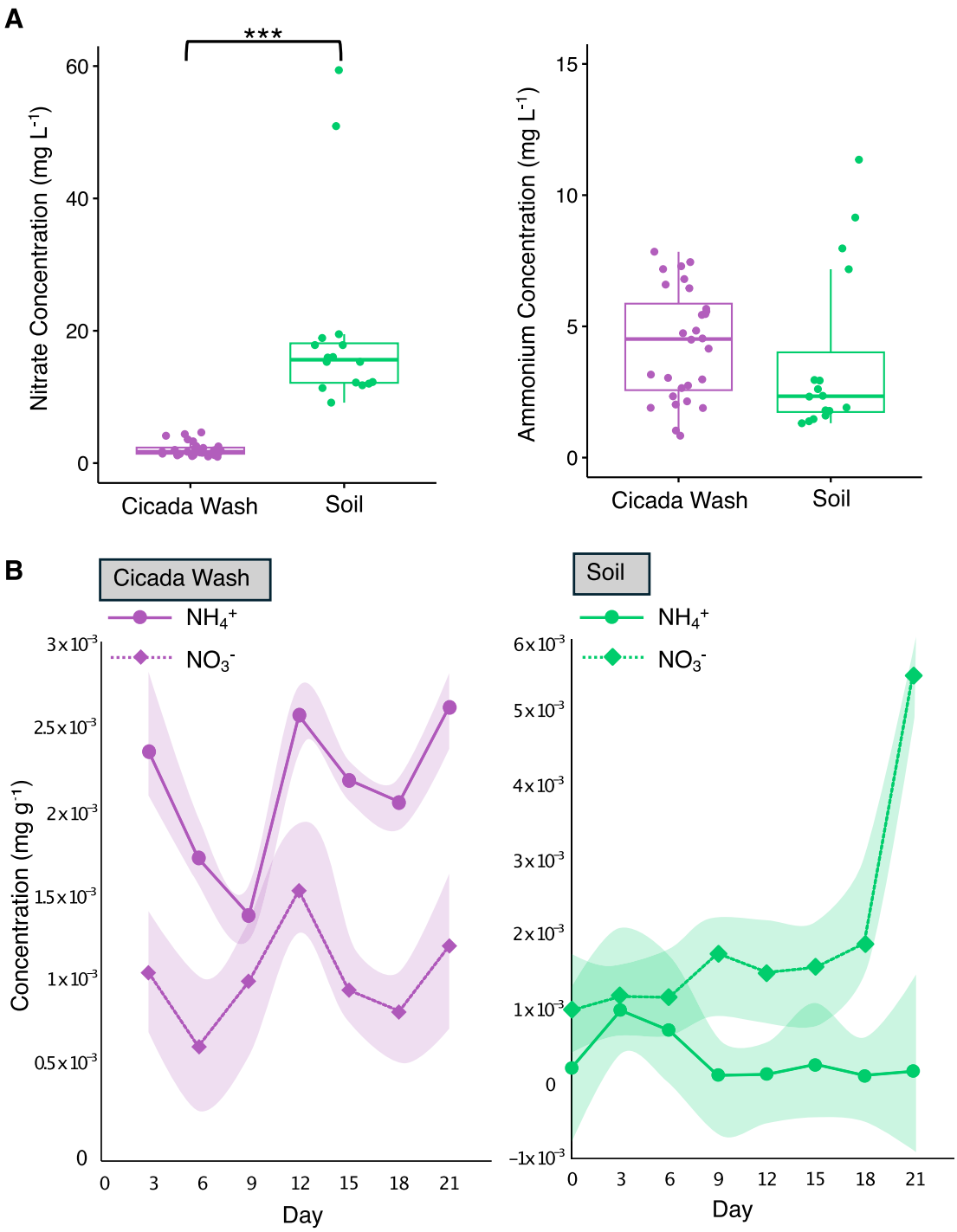

Fig. 6. A. Mean concentrations of nitrate and ammonium (mg g^{-1}) from soil and cicada washes over 21 days. **B.** Concentrations of nitrate and ammonium (mg g^{-1}) measured every 3 days for 21 days from soil and cicada washes. Concentrations were measured using a FLUOstar® Omega filter-based multi-mode microplate reader. Shading indicates standard error. $N = 32$.

have limited the rate of carcass decomposition, leading to observation of no significant difference in gas emissions between the "low" and "high" cicada treatments in the field experiments.

Further quantification of microbial activity is required to determine the precise mechanisms of N cycling and increased gas emissions but this work is nevertheless an important examination of this ecosystem scale phenomenon with consequences for understanding of forest biogeochemistry and climate modelling. We have determined that the interface between cicada carcasses and soil surfaces is a critical unexplored niche for increased biogeochemical cycling and subsequent gas emissions following periodical cicada emergence. This effect appears to be highly localised to the soil surface in direct contact with cicada carcasses, as the microbiome community of surrounding soil did not exhibit the same variation. From this work we suggest that increased gas fluxes as a result of cicada emergence and decomposition are due to microbial nutrient cycling at the interface between cicada carcasses and the soil surface (Fig. 7). In this conceptual figure, the contribution of cicada carcasses to N cycling does not end with carcass decomposition and N mineralisation increasing N availability to plants (Yang, 2004). Rather, organic N enters the soil from cicada decomposition, undergoes transformations in

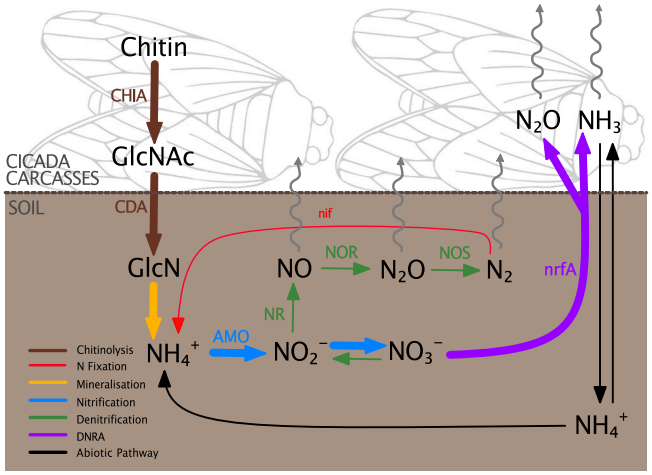

Fig. 7. Conceptual figure showing potential microbial mechanisms in the soil and on cicada carcass surfaces behind increased ammonia (NH_3) and nitrous oxide (N_2O) gas fluxes from forest soils following periodical cicada emergence. Chitin-rich cicada carcasses are broken down by chitinolytic microbes as a source of organic nitrogen (N) that enters the soil. In the soil, N undergoes mineralisation to ammonium (NH_4^+), followed by nitrification and denitrification processes. Nitrification produces nitrate (NO_3^-), which can produce nitric oxide (NO), N_2O, and dinitrogen gas (N_2) via denitrifcation. NO_3^- can also be reduced to NH_3 via dissimilatory nitrate reduction to ammonia (DNRA) and lost as gas along with the by-product N_2O, or returned to the soil as NH_4^+. N_2 can be lost from the system or microbially fixed to feedback into the N cycle, CHIA = chitinase; CDA = chitin deacetylase; AMO = ammonia monooxygenase; NR = nitrite reductase; NOR = nitric oxide reductase; NOS = nitrous oxide reductase; nrfA = nitrite reductase (DNRA); nif = nitrogenase. Thicker arrows indicate the suggested dominant processes that lead to increased emissions of N gases.

the soil, and then feeds back to N cycling processes by microbes both in the soil and on cicada carcass surfaces, resulting in increased gas emissions than would usually be produced without such a large input of organic N.

4.1. *Cicada decomposition fuels heterotrophic nitrification*

Chitin is highly abundant in nature and is a structural element in fungi, algae, and insects. Interactions of chitin with C and N cycling are well studied, and it is suggested that the turnover rate of chitin is high due to a lack of significant environmental accumulation (Beier and Bertilsson, 2013). Chitin degradation is primarily controlled by bacterial taxa, including in soils, however fungal taxa also contribute (Beier and Bertilsson, 2013; Kielak et al., 2013; Manucharova and Vlasenko, 2011). The degradation of chitin by soil microbes is a well-known and significant contributor to terrestrial C and N cycling (Hui et al., 2020). It has been reported that many diverse chitinolytic microbes are present in soil, and so chitin can be degraded under differing redox conditions as a result of soil properties and environmental conditions (Wieczorek et al., 2014). In stream ecosystems, cicada carcasses are rapidly colonised by microbes, providing a large readily used resource pulse and increasing respiration rates (Menninger et al., 2008). Microbial biomass is also rapidly increased following cicada carcass addition as "litter" to the soil (Yang, 2004). Studies have found that chitin is decomposed and incorporated into microbial biomass faster than other lower molecular mass substrates such as glucose and cellulose (Huang et al., 2024; Martinović et al., 2022), suggesting the large input of chitin from cicada carcasses would be rapidly utilised by the microbial community. The extent of the ecological impact of "pulse events", such as periodical cicada emergence is determined by rate and duration, spatial and temporal distribution, and the biotic response to the event (Jentsch and White, 2019). However, there has been little discussion in the current literature on the effects of chitin input at the scale of a periodical cicada emergence. We suggest cicada carcass decomposition represents an understudied, yet potentially critically important agent for rapid and expansive microbial growth and activity, including in the less well-studied process of HN, with consequences for soil surface biogeochemistry.

Chitin from cicada carcasses can either be directly hydrolysed to GlcNAc which is then broken down to GlcN, or first deacetylated to chitosan and subsequently hydrolysed to GlcN (Einbu and Vårum, 2008). The dominant mechanism is likely to be the former, as chitosan is not as abundant in the environment and microbial activity was stimulated slower by chitosan than by chitin in a supplementation experiment by Wieczorek et al. (Wieczorek et al., 2014). The most abundant chitinolytic taxa we found was *Serratia marcescens*, a bacteria with an extensively studied chitinase system. *S.marcescens* produces multiple chitinase enzymes with distinct roles in the degradation of chitin, which may lead to more efficient chitin utilisation (Beier and Bertilsson, 2013). We also identified *Paenibacillus chitinolyticus*, a prolifically chitinolytic bacteria first isolated from forest soils (Liu et al., 2020). The presence of these taxa in soils, but primarily on cicada carcass surfaces, indicates the capacity for the microbial community to degrade chitin from cicada carcasses and utilise the C and N content. As the cicada carcasses used in this study were collected from the ground, they may have experienced rapid microbial colonisation prior to collection, leading to elevated abundance of chitinolytic taxa in the carcass surface necrobiome seen on day 0 of the time series experiment that originated from soil. In a study by Martinovic et al. (Martinović et al., 2022), it was found that the respiration of C substrates such as cellulose, glucose, and citric acid plateaued after 7 days, however in our experiments, the respiration of chitin continued to increase throughout the 21 day experiment. Cicada carcasses acting as a natural "fertiliser" rather than other organic matter sources may therefore have a longer-term effect on soil respiration and nutrient cycling than other resource inputs from insects (Lovett et al., 2002), making the subsequent gas pulses more significant.

The contribution of HN to overall nitrification is variable and depends on pH, O_2 availability, and organic matter content (Martikainen, 2022). Usually, the relatively low availability of organic matter as substrates for HN limits the contribution of this process to soil nitrification as a whole (Cao et al., 2023; Martikainen, 2022). However, in systems such as after cicada emergence and death where there has been a large input of organic matter, this limit could be alleviated, making HN more significant. In soils used in this experiment, we identified numerous microbial taxa that carry out HN including *Pseudomonas* (Obaton et al., 1968; Trung Tran et al., 2019), *Bacillales* (Mendoza et al., 2019), and several species of *Streptomyces*, which are known to carry out HN in soils rich in organic matter (Grzyb et al., 2021; Saadoun et al., 2017) as is likely to be the case following cicada carcasses inputs. High respiration rates indicate active decomposition of organic matter, and the higher respiration rates measured from cicada carcass-amended soils in this work demonstrate that carcasses are acting as a significant source of organic matter for microbes, including those capable of carrying out HN. Alongside HN, autotrophic nitrification also occurred in this system, though we detected far more ammonia- and nitrite-oxidising taxa in the soil microbiome relative to the cicada necrobiome. Autotrophic nitrifiers generally grow slowly, and our results suggest that their low abundances in carcasses might result from being outcompeted for NH_4 by heterotrophs with the capacity for HN that favour the N-rich conditions of the necrobiome (Kindaichi et al., 2004). The positive correlation between NO_3^- concentration in soil microbiome and corresponding increase in abundance of nitrification taxa in the cicada necrobiome further demonstrates the importance of the interface between decaying carcasses and the soil surface to the potential pathway of increased gas fluxes identified in this work. Among the ammonia-oxidising community, AOB dominated the soil microbiome and AOA dominated the cicada necrobiome, suggesting that different taxa are able to take advantage of the particular niches created during organic matter decomposition, leading to increased N gas production.

4.2. DNRA likely contributes significantly to N_2O and NH_3 production in cicada-disturbed soils

Overall soil contribution to global N_2O fluxes is approximately 70% and although agricultural soils are the largest source of N_2O, forest soils also contribute significantly by emitting 2.4–5.7 Tg yr^{-1}.[18] Emissions of N_2O from forest soils are dependent on a variety of climatic and environmental factors, including temperature, precipitation, soil texture, redox conditions, and soil C:N content.[19] Higher C:N has been reported to favour DNRA over denitrification (Wan et al., 2015), and the high C:N of cicada carcasses measured in this work could be a factor contributing to an increase in DNRA. Soils are also considered to be the most significant source of global NH_3 emissions, particularly those fertilised for agriculture.[20] In our time series experiment, abundance of DNRA taxa peaked between day 15–18 after cicada addition to soil, as did N_2O and NH_3 fluxes. While our correlative evidence strongly implicates DNRA, we acknowledge that conventional denitrification and nitrifier denitrification also contribute to observed emissions. Direct rate measurements using ^{15}N tracing techniques would be needed to definitively quantify the relative contribution of each pathway.

We suggest the majority of the excess N input from cicada carcasses is cycled by the soil microbiome, then returns as NO_3^- to the necrobiome for denitrification and DNRA. During denitrification, NO and N_2O can be lost from the soil due to incomplete reduction, particularly under the high NO_3^- conditions that occur in this system that favour NO_3^- and NO_2^- reduction over later denitrifiying steps. NO_3^- that undergoes complete denitrification to N_2 could then be fixed by N-fixing microbes and be retained with in the soil N cycle. DNRA taxa are less sensitive to oxygenated conditions and fluctuating redox conditions, such as those that may be created by cicada emergence tunnels, than denitrifying taxa (Pett-Ridge et al., 2006).

There was a high abundance of *S. marcescens* in the cicada carcass necrobiome. *S. marcescens* possesses both chitinolytic and DNRA capabilities, creating an elegant ecological scenario where the bacterium degrades the chitin substrate and then opportunistically utilises NO_3^- for DNRA (Hamada and Soliman, 2023) when it becomes available. This dual functionality may explain the unusually high abundance of DNRA-capable taxa after cicada carcass deposition. However, we cannot rule out that different microbial taxa are responsible for these sequential processes, or that conventional denitrifiers also contribute significantly to the observed N_2O production. Further investigation using isotopic approaches to determine the precise activity of *S. marcescens* and other taxa throughout cicada decomposition could help elucidate the relative contribution of each microbial process to increased soil gas emissions.

4.3. Ammonia oxidation in soils driven by AOB after cicada nutrient input

Temporal patterns of NH_4^+ and NO_3^- concentrations demonstrate that cicada carcasses are acting as a source of organic N for soils. After 3 days there was a large increase in soil NH_4^+ concentration suggesting GlcN has been mineralised to NH_4^+ and is now present in the soil for further microbial processing. An increase in soil NO_3^- concentration after 15 days suggests NH_4^+ has been nitrified and is then available as a substrate for denitrification and DNRA taxa to produce the pulse of N_2O seen from these samples at around the same time (Fig. 2). As discussed above, NH_4^+ can be lost via NH_3 volatilisation under the alkaline microsite conditions at the carcass-soil interface. However, the majority of NH_4^+ will be nitrified, as there are favourable conditions for increased microbial activity (Ti et al., 2021). The initial step of nitrification, ammonia oxidation, is carried out by bacterial and archaeal taxa encoding the amoA gene and it has been reported that the addition of chitin to soil stimulates ammonia oxidising microbial activity (Zhang et al., 2024). In many soils, abundance of AOA taxa is significantly higher than AOB taxa and NH_3 is primarily oxidised by AOA, however recent studies have found that production of some gaseous N cycle products and by-products, is driven mainly by AOB (Mushinski et al., 2019; Purchase et al., 2023). In this study, abundance of AOA taxa was higher than AOB yet the activity of the bacterial *amoA* gene was not significantly higher, suggesting there is no significant dominance between AOA or AOB. We suggest nitrifier denitrification by ammonia oxidisers is a highly active process in the soil samples, as NO_3^- concentration was negatively correlated with the soil abundance of AOB. It has been reported by Wrage-Mönnig et al. (Wrage-Mönnig et al., 2018) that in some soil systems, nitrifier denitrification may be responsible for up to 100% of N_2O emissions. Although other processes such as conventional denitrification and DNRA are the more dominant N_2O forming processes in these samples, nitrifier denitrification also contributes to both N_2O and N_2 production. Cicada carcasses are on the soil surface and therefore the input of organic N and subsequent enhanced microbial cycling of N is likely to be constrained to the aerobic surface soils, where nitrifier denitrification will dominate over conventional denitrification (Wrage et al., 2001). The process of cicada emergence may also aerate the soil through the formation of emergence holes, which supports nitrifier denitrification as an important source of N_2O. Nevertheless, the surface location of cicada carcasses means that aerobic processes like nitrifier denitrification may dominate over strictly anaerobic denitrification in the upper soil layers, while DNRA may be most active in anoxic microsites created by intense microbial respiration at the carcass-soil interface.

4.4. Spatiotemporal scaling of cicada-induced soil emissions

Periodical cicada emergence occurs over a large area of North America and as the emergence years of active 13- and 17-year broods are staggered, there are cicadas emerging almost every year between late April and early June. The hotspot of N_2O and NH_3 emissions described here as a consequence of nutrient input from cicada carcasses could represent a massive, yet highly predictable force contributing to climate change. Although N deposition to the eastern U.S. has decreased significantly since 1990, it still remains higher than pre-industrial levels, and annual N deposition in 2021 was up to 20 kg-N ha^{-1} in some regions of the U.S., but was approximately 6–8 Kg-N ha^{-1} on average (United States Environmental Protection Agency, 2021). The amount of N available from cicada carcasses is relatively little compared to annual N deposition, ~ 2.5 kg-N ha^{-1} yr^{-1} using estimates of ~200 mg dry cicada weight and a mean N content of 8.78% of each cicada as quantified in this work. However, atmospheric N deposition occurs on a global scale, whereas cicada deposition is highly localised meaning cicada contribution may be less impactful globally on an annual basis, though still ecologically significant during emergence years. A study by Krichels et al. (Krichels et al., 2023) reported a range of annual NH_3 emissions from U.S. forest soil between 6.74 and 212.59 mg-N m^{-2}, depending on soil and environmental conditions. Annual N_2O emissions have been quantified as ~20 mg-N m^2 from forest soils (Peng et al., 2024). The majority of NH_3 and N_2O emitted from soils is from agricultural areas, with forest soils contributing less to the annual total. For example, ~9% of total US soil N_2O emissions each year between 2010 and 2019 were attributed to forest soils (Lu et al., 2022). Per cicada N content in this work was approximately 17.5 mg-N. N use efficiency varies significantly due to soil properties, but on average is 55%–60% of organic N is taken up by microbes during decomposition (Mooshammer et al., 2014). Assuming an efficiency of 55%, during one emergence event with an emergence density of 250 insects m^{-2}, cicadas may contribute ~4375 mg-N m^{-2} to the soil. From our flux data we estimate combined N losses as N_2O and NH_3 gas to be ~0.027% of the cicada N input, which would cumulate as between 0.33 and 0.84 mg-N m^{-2} h^{-1} and a mean of 0.53 mg-N m^{-2} h^{-1} lost as N gases during decomposition of cicada carcasses following a single emergence event. This would be equivalent to a short-term ~35-fold increase on average mg-N m^{-2} $year^{-1}$ lost as N_2O and NH_3 from US forest soils. Although some assumptions are necessary to calculate these values, they nevertheless demonstrate that a single cicada emergence event could contribute emissions of NH_3 and N_2O

equivalent to a significant proportion of current annual estimates but are not yet included in predictive models. Due to the spatial heterogeneity of periodical cicada emergences, further research in this area should take into account the effects of a wider range of cicada carcass densities on the microbiology and biogeochemical cycling of soil.

Studies on the longer-term ecosystem effects of periodical cicada emergence are few. Research on mass mortality events in general has reported that fertilisation effects may be short-lived, but could have longer term impacts on higher trophic levels within the ecosystem (Fey et al., 2019). In forests that experience cicada emergence events more often, legacy effects may mean that the composition of the soil microbial community has changed to be able to utilise these nutrient impulses more effectively. In a study by Yuan et al. (Yuan et al., 2022), it was demonstrated that bacterial taxa are more likely to be favoured by less frequent nutrient pulses, whereas fungal taxa are favoured by repeated or continuous input. Therefore, the response of forest soils to cicada emergence may also be dependent on the frequency of emergence events and will differ geographically. The combined effects of N enrichment and climate change may have implications for forest management strategies. As climate change progresses, invasive species that are better adapted to warmer temperatures and increased N conditions may be able to take advantage of the organic N input from cicada carcasses and begin to outcompete native plants, leading to shifts and losses of biodiversity (Poland et al., 2021; Valliere et al., 2022). Populations of periodical cicadas may themselves be impacted by climate change, affecting their ability to emerge and reproduce successfully. This will have consequences for trophic cascades across entire forest ecosystems (Getman-Pickering et al., 2023). It is therefore crucial that biogeochemical cycles of forest soils and the wider ecosystems are better understood, to ensure resilient and biodiverse forests for the future.

4.5. *Future research directions*

Given these complex ecological interactions and the potential impacts of climate change on both cicadas and forest ecosystems, several key research priorities emerge to strengthen our mechanistic understanding and predictive capacity. Direct quantification of N cycling rates using ^{15}N isotope tracing would allow precise partitioning of N_2O production among DNRA, denitrification, and nitrifier denitrification. Microscale measurements of pH and redox conditions at the carcass-soil interface would validate our proposed mechanisms for NH_3 volatilisation and anaerobic N cycling. Additionally, examining multiple broods across different forest types and emergence frequencies would test the generality of our findings and reveal potential legacy effects of repeated emergences on soil microbial community composition and biogeochemical function. Such studies would refine predictions of cicada-induced greenhouse gas emissions for inclusion in regional N budgets and climate models.

5. Conclusions

By studying the biogeochemical consequences of the 2021 Brood X emergence in IN, we have identified significant hotspots of gas emissions from soil following periodical cicada emergence, with emissions of N_2O and NH_3 at ~0.5 mg-N m^{-2} h^{-1} representing an estimated 35-fold short-term increase over annual averages. We hypothesised that cicada carcasses would serve as a large input of N to forest soils, stimulating N cycling microbial activity and leading to increased emissions, and our data support this hypothesis. The interface between cicada carcasses and soil surfaces represents a critical unexplored niche for biogeochemical cycling. The cicada necrobiome, particularly chitinolytic and DNRA-capable taxa such as *Serratia marcescens*, works in concert with soil microbial communities carrying out nitrification, denitrification, and nitrifier denitrification to drive greenhouse gas emissions. Alongside conventional denitrification in the soil, we identified potential for nitrifier denitrification and DNRA microbes that inhabit the cicada necrobiome to contribute significantly to the increased emissions of N_2O and NH_3, working in concert with other N-cycling processes. Although inputs of N to soil from cicada carcasses may be relatively small compared to annual N deposition, particularly from anthropogenic sources, hotspots of greenhouse gases resulting from cicada emergence may be a highly predictable contributor to climate change that is currently understudied and not well understood. As climate change progresses, the combined effects of N enrichment and altered temperature regimes may have implications for forest management strategies, cicada population dynamics, and trophic cascades across entire forest ecosystems. These data provide novel insights into the biogeochemical consequences of this unique ecosystem disturbance and will encourage further investigation into the complex microbiological activity in the soil microbiome and cicada necrobiome that occurs following periodical cicada emergence.

CRediT authorship contribution statement

Megan L. Purchase: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Richard P. Phillips:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jonathan D. Raff:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Amy I. Phelps:** Investigation. **Elizabeth Huenupi:** Writing – review & editing, Resources, Investigation. **Ryan M. Mushinski:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.106813.

Data availability

Data are available at doi: 10.17632/kk92p96r2x.1. Sequences can be found at NCBI under BioProject PRJNA1297665. Code is available at https://github.com/MeganPurchase/Cicada_Fluxes_25.git.

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