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RESEARCH ARTICLE

Nitrogen Deposition Weakens Soil Carbon Control of Nitrogen Dynamics Across the Contiguous United States

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ABSTRACT

Anthropogenic nitrogen (N) deposition is unequally distributed across space and time, with inputs to terrestrial ecosystems impacted by industry regulations and variations in human activity. Soil carbon (C) content normally controls the fraction of mineralized N that is nitrified ($f_{nitrified}$), affecting N bioavailability for plants and microbes. However, it is unknown whether N deposition has modified the relationships among soil C, net N mineralization, and net nitrification. To test whether N deposition alters the relationship between soil C and net N transformations, we collected soils from coniferous and deciduous forests, grasslands, and residential yards in 14 regions across the contiguous United States that vary in N deposition rates. We quantified rates of net nitrification and N mineralization, soil chemistry (soil C, N, and pH), and microbial biomass and function (as beta-glucosidase (BG) and *N*-acetylglucosaminidase (NAG) activity) across these regions. Following expectations, soil C was a driver of $f_{nitrified}$ across regions, whereby increasing soil C resulted in a decline in net nitrification and $f_{nitrified}$. The $f_{nitrified}$ value increased with lower microbial enzymatic investment in N acquisition (increasing BG:NAG ratio) and lower active microbial biomass, providing some evidence that heterotrophic microbial N demand controls the ammonium pool for nitrifiers. However, higher total N deposition increased $f_{nitrified}$, including for high soil C sites predicted to have low $f_{nitrified}$, which decreased the role of soil C as a

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predictor of $f_{\text{nitrified}}$. Notably, the drop in contemporary atmospheric N deposition rates during the 2020 COVID-19 pandemic did not weaken the effect of N deposition on relationships between soil C and $f_{\text{nitrified}}$. Our results suggest that N deposition can disrupt the relationship between soil C and net N transformations, with this change potentially explained by weaker microbial competition for N. Therefore, past N inputs and soil C should be used together to predict N dynamics across terrestrial ecosystems.

1 | Introduction

The rise in agricultural production and fossil fuel combustion during the 20th century increased nitrogen (N) emissions and, consequently, atmospheric N deposition to terrestrial ecosystems across the globe (Fixen and West 2002; Gruber and Galloway 2008). This deposition caused widespread negative environmental impacts, including elevated nitrate (NO₂⁻) leaching and nitrous oxide (N₂O) emissions (Schlesinger 2009; Vitousek et al. 1997). As such, legislative efforts were imposed to curb these atmospheric N inputs. Air quality regulations in the United States cut emissions of nitrous oxides (NO₂) by 41% from 1990 to 2010 (Li et al. 2016), reducing inorganic N deposition by 0.11 kg N ha⁻¹ year⁻¹ in the eastern United States during this period (Ackerman, Millet, and Chen 2019). Although inorganic N deposition rose 8% globally from 1984 to 2016, regions like Europe and Central Indo-Pacific had downward trends in N deposition (Ackerman, Millet, and Chen 2019). The drop in anthropogenic N deposition, combined with elevated atmospheric CO₂ concentrations, can reduce N availability for plants and soil microbes (Garten, Iversen, and Norby 2011; Norby et al. 2010), with emerging evidence suggesting unmanaged ecosystems worldwide are returning to N-limited states (Mason et al. 2022; McLauchlan et al. 2017). The long-term decline in reactive N deposition likely affects ecosystem functions, including decomposition and nitrification. Yet, much remains to be discovered about the variable effects of N deposition across space and time on the microbially mediated N cycle and its relationship with soil carbon (C).

Aside from bioavailable N derived from atmospheric deposition, terrestrial N availability is controlled by soil microbial communities. Heterotrophic soil microorganisms use extracellular enzymes to break down soil organic matter (SOM) for energy and materials (Burns 1982; Sinsabaugh 1994). Microbial enzyme production facilitates N mineralization in soils by converting simple organic N compounds from plants and microbes (including N-fixing bacteria) to ammonium (NH_4^+) . Ammonium may then be oxidized by chemolithoautotrophic nitrifiers into NO_3^{-} , with the potential to be leached into adjacent waterways or lost as the potent greenhouse gas N₂O (Rao et al. 2014; Wallenstein et al. 2006). Both ammonium and nitrate can be immobilized by heterotrophic microbes or taken up by plants to meet N demands (Melillo et al. 1989; Soong et al. 2020). Additionally, larger soil C pools increase heterotrophic N demand to maintain their C:N stoichiometry (Cleveland and Liptzin 2007; Redfield 1958; Schimel and Weintraub 2003), leading to increased N mineralization and immobilization rates but decreased nitrification rates. While site-dependent variables like soil moisture and NH4+ do drive nitrification rates, it has been shown at local (Keiser, Knoepp, and Bradford 2016) and continental (Gill et al. 2023) scales from laboratory incubations and field-based assays that soil C content determines whether or not mineralized N (NH_4^+) is nitrified. Specifically, the fraction of mineralized N that is nitrified ($f_{\rm nitrified}$) is lower under high soil C conditions, likely driven by heterotrophic N immobilization (Elrys et al. 2021), reducing NH₄⁺ availability for nitrifiers. As a result, net N mineralization and nitrification rates can become decoupled under high microbially available C conditions. However, large pulses of external N inputs (e.g., fertilizer application) may exceed heterotrophic N demand, resulting in high nitrification rates and $f_{\rm nitrified}$ across an array of soil C concentrations as competition for N eases between microbial heterotrophs and nitrifiers (Aber et al. 1998; Yuan et al. 2019). While it is expected that an increase in N availability will increase nitrification rates, the effects of atmospheric N deposition on the role of soil C in mediating $f_{\rm nitrified}$ have not been resolved.

To identify how the activity of soil microorganisms shifts with N availability, we can quantify changes in their functional attributes that characterize their C- and N-cycling potentials, including extracellular enzyme activity (EEA). Field experiments show that N-acquiring enzyme activity declines with concomitant increases in C-acquisition enzyme activity under experimental Nfertilization rates ranging from 30 to 100 kg N ha⁻¹ year⁻¹ (Ajwa, Dell, and Rice 1999; Saiya-Cork, Sinsabaugh, and Zak 2002; Zeglin et al. 2007). These studies suggest that experimental N fertilization rates-typically greater than realized N deposition rates-lower the enzymatic investment to acquire N by soil microbes, indicative of lower microbial N limitation. In addition, decomposition measures, such as litter mass loss and microbial respiration, decrease with experimentally higher N availability (Craine et al. 2007; Knorr, Frey, and Curtis 2005; Treseder 2008). A decline in decomposition due to decreased microbial N limitation may increase SOM pools as heterotrophic microbes rely less on this organic pool for N (Bowden et al. 2019). Yet, studies report contrasting effects of N-addition on soil C stocks. In forests, chronic N fertilization increases the stock of organic C in the topsoil layer (Frey et al. 2014), whereas soil C stocks do not change in grassland N-addition experiments (Keller et al. 2022). This distinction between forest and grassland soils not only reveals key differences in soil microbial communities between these systems (Carson et al. 2019; Edwards et al. 2011; Frey et al. 2004) but also the importance of understanding what mechanisms govern microbial responses to higher N availability across ecosystems, including edaphic properties known to affect the N cycle, such as soil pH (Kemmitt et al. 2006; Riggs and Hobbie 2016).

As N deposition decreases in response to environmental regulations, ecosystem recovery may lag behind this decline (Gilliam et al. 2019; Stevens 2016) given that ecosystems retain exogenous N in plant and soil pools (Lovett and Goodale 2011). From the few field experiments where high rates of N fertilization have ceased and observations have continued, N mineralization rates have been shown to remain elevated for over

5 years compared to never-fertilized controls (Clark et al. 2009; O'Sullivan et al. 2011), but nitrification rates can recover in 1 year (Nieland et al. 2021). Yet, these experiments coincide with the slow, multidecadal decline in N deposition in many regions and concurrent rise in atmospheric CO₂ concentrations, with studies without experimental N fertilization reporting decreases in plant tissue N concentrations and natural abundance $\delta^{15}N$ values, ecosystem N-cycling rates, and aquatic N exports from watersheds (Groffman et al. 2018; Penuelas et al. 2020; Sabo et al. 2020). The observational N fertilization studies instead suggest that legacies of anthropogenic N deposition may not be realized in natural systems because contemporary atmospheric chemistry, specifically CO2 fertilization, decreases N availability and increases overall ecosystem N demand (Garten, Iversen, and Norby 2011; Norby et al. 2010). With limited experimental data matching low and variable rates of N deposition, it remains uncertain as to how soil microbial communities respond functionally to decreased anthropogenic N deposition across diverse ecosystems (Lamarque et al. 2013).

The COVID-19 pandemic prompted a sudden drop in human activity around the globe as 2020 lockdowns restricted work and outdoor activities in an attempt to slow the spread of SARS-CoV-2 (Alfano and Ercolano 2020), the virus that causes COVID-19. Consequently, 2020 vehicular traffic and industry activity decreased (Liu and Stern 2021) with increases in select air quality metrics (i.e., PM_{2.5}, PM₁₀, and NO₂; Yang et al. 2022) and avian and beach flora and fauna abundances across urban ecosystems (Schrimpf et al. 2021; Soto et al. 2021). However, the effects of the COVID-19 pandemic on terrestrial biogeochemistry are not known, despite reported declines in atmospheric N deposition (Berman and Ebisu 2020; Le Quéré et al. 2020). This "anthropause" (Rutz et al. 2020) presents the opportunity to investigate whether a short-term decrease in N deposition during 2020 alters the relationships among soil C, net N mineralization, and net nitrification and the strength of soil C as a driver of $f_{\text{nitrified}}$.

Taking advantage of both the decline in rates of atmospheric N deposition during the COVID-19 pandemic and the range in background atmospheric N deposition across the contiguous United States, we examined under laboratory conditions if N deposition alleviates soil C-controlled competition for N between microbial heterotrophs and nitrifiers. We sampled 14 regions (with multiple sites per region) experiencing variable N deposition rates (annual means: 3.2–11.7 kgNha⁻¹year⁻¹), and measured soil net nitrification and N mineralization rates, EEAs, active microbial biomass with substrate-induced respiration (SIR), and soil chemistry (soil C, N, and pH). We hypothesized (H1) that high C soils with high background rates of atmospheric N deposition exhibit higher net nitrification rates and $f_{\rm nitrified}$ than high C soils with low background N deposition because NH4+ supplied through deposition would alleviate NH₄⁺ limitation of nitrifiers induced by immobilization (Figure 1a,b). Initiating the study during the COVID-19 pandemic, we leveraged this natural experiment to discern if a short-term dip in contemporary N deposition decreased $f_{\text{nitrified}}$. We hypothesized (H2) that a temporary decrease in N deposition strengthens the role of soil C in regulating net N transformation rates and $f_{\rm nitrified}$, resulting in a decrease in net N transformation rates and $f_{\rm nitrified}$ because microbial immobilization would drive NH_4^+ limitation

for nitrifiers at sites where background N deposition rates are typically at intermediate or high levels (Figure 1c).

2 | Methods

2.1 | Study Sites and Sample Collection

We sampled soils from 14 regions across the United States that varied in rates of atmospheric N deposition and climate (Table S1). Each region included individual sites that captured a range of vegetation and land uses, including forest, grassland, and residential yards, for a total of 39 sites. At a minimum, each region had one natural ecosystem reflecting the area's dominant ecosystem type and one residential yard. We included yards because they offer a relatively similar comparison, in terms of vegetation, across the 14 regions and climates, and most yards shared similar management across regions (Table S1). We classified non-yard sites into coniferous forest, deciduous forest, grassland, scrub, and oak-palmetto forest ecosystems based on vegetation and climate. Thirty-year mean annual precipitation (MAP) and temperature (MAT) were estimated for each site using the closest weather station in the National Weather Service Cooperative Network (NWS COOP). Monthly precipitation (PPT) and potential evapotranspiration (PET) during 2013-2021 were calculated to identify climate (i.e., mesic or xeric) using daily precipitation, daily maximum and minimum temperatures, and latitude (for solar radiation; Allen et al. 1999). A PPT:PET ratio of less than 1 was defined as a xeric climate (Knapp et al. 2008).

Samples were collected by taking the top 10 cm of mineral soil with a trowel to fill approximately a quart-size (0.95-L) sterile bag four times at each site in 2020. These collection times were selected to correspond with changes in national activity due to COVID-19 restrictions in 2020 (with increasing human activity across time): April (stay at home), May (partial reopening), June, and August. Each sample was split in half with one subsample immediately frozen and the other air-dried. Once COVID-19 restrictions lifted, dried and frozen samples were shipped to the University of Massachusetts Amherst where frozen samples remained at -20°C until processed. For this study, we analyzed soils from the first and fourth collections to capture the timepoints with the widest range in COVID restrictions and potential for contrasting N deposition rates. Because some sites included replicated plots, samples from plot replicates were processed separately and then averaged within a site for data analysis. Samples were collected at a subset of the sites a year after the initial collections in 2021 to determine if changes in local deposition levels affected soil microbial functions. While the seven sites resampled were collected from the Northeastern United States because of their proximity to the University of Massachusetts Amherst, we only used these sites to compare soil functional parameters between a year with reduced human activity (2020) and a year with closer to normal activity (2021). For these samples, we kept plot replicates separate for data analysis.

2.2 | Atmospheric N Deposition Estimates

Annual total (wet + dry) N deposition and wet NH_4^+ deposition were estimated for each site using model outputs



FIGURE 1 | Conceptual model of hypotheses. (Top panel) Under low N deposition (a), net nitrification and N mineralization rates are expected to couple ($f_{nitrified} = 1$) in low soil C conditions due to reduced competition for NH₄⁺ between microbial heterotrophs and nitrifiers. In contrast, high soil C facilitates strong competition, resulting in NH₄⁺ limitation for nitrifiers and decoupled net nitrification and N mineralization rates ($f_{nitrified} = 0$). As background N deposition increases (b), competition between microbial heterotrophs and nitrifiers for NH₄⁺ weakens as nitrifiers can switch their source for NH₄⁺ resulting in higher net nitrification rates in high C soils. (c) A short-term dip in N deposition because of a COVID-19 response to reduced vehicle emissions is predicted to decrease net nitrification rates, lowering $f_{nitrified}$, particularly for soils with high C content as heterotrophic immobilization increases.

detailed in Schwede and Lear (2014). Grids from the National Atmospheric Deposition Program's (NADP) National Trends Network (NTN) (version 2023.01; https://nadp.slh.wisc.edu/ committees/tdep/) were accessed on November 14, 2023, and uploaded to RStudio V2023.12.1+402 (Posit team 2024) using R package raster (Hijmans 2023). We collected deposition data from online databases covering 2013-2021 (pre-study years plus study period). While an expected decrease in deposition induced by the COVID-19 pandemic inspired the current study, preliminary data analysis indicated that annual N deposition rates declined in 2018 and through 2020, before rebounding in 2021. Because we wanted to investigate both background and contemporary N deposition effects motivated by the COVID-19 restrictions, we defined background N deposition as 2013-2017 before N deposition began to decline. Using the 2013-2017 values, we averaged annual total N deposition estimates for each of the 39 sites to define "low" (2013-2017 mean site total N deposition range: 3.18-6.93 kg N ha⁻¹ year⁻¹), "intermediate" (7.53–8.45 kg N ha⁻¹ year⁻¹), and "high" (8.56– 11.67kgNha⁻¹year⁻¹) background rates of N deposition from the 33rd and 66th percentiles of the mean rates of N deposition. We binned background N deposition into ranks because

preliminary analyses of 2013–2017 mean site N deposition rates showed three distinct groups, which approximately aligned with the 33rd and 66th percentiles. Each N deposition rank included 13 sites. Average annual $\rm NH_4^+$ deposition was also calculated for each site.

The decline in human activity from the COVID-19 pandemic presented unique challenges to quantifying real-time wet N deposition declines because many NTN stations were closed during this time. Therefore, we used the automated United States Environmental Protection Agency's (EPA) Clean Air Status and Trends Network (CASTNET) (https://epa.gov/castnet/) to gather particulate (dry) N deposition data since CASTNET stations were not disrupted during the lockdown period of spring 2020. We extracted weekly CASTNET dry N concentration data from 2013 to 2021 on February 14, 2024, from six stations. These stations were selected because of their proximity to sampling locations and variation in total background N deposition rates (Figure S1). With the CASTNET data, we first calculated dry N deposition flux using deposition velocities reported by Holland et al. (2005) and then added the fluxes to report annual cumulative dry N deposition. As with total N deposition, 2013-2017

served as background years to calculate the 95% confidence interval for dry N deposition. Dry N deposition in 2018-2021 that fell outside the confidence intervals was considered significantly different from background N deposition at $p \le 0.05$. The fraction of annual total N deposition deposited as dry N, based on the 2013-2017 NTN and CASTNET data, ranged from (mean \pm standard deviation) 10.4% \pm 2.2% in northern Montana to $61.7\% \pm 4.9\%$ in southern California (overall mean: 22.4%). Thus, we are somewhat limited in our inference about the potential change in total N deposition during the early part of the COVID-19 pandemic due to missing wet N deposition data in 2020. However, dry N deposition serves as a good indicator for vehicular activity since dry N deposition is higher in urban sites (Bettez and Groffman 2013) and deposition rates decline exponentially away from roads (Redling et al. 2013) [with some notable exceptions (Rocci, Cotrufo, and Baron 2023)].

2.3 | Soil Chemistry

Air-dried soils were sieved to 2 mm and then pulverized using a CertiPrep 8000-D Mixer mill (Spex, Metuchen, NJ, USA), and total C and N from two milled analytical replicates were quantified using a Carlo Erba NA1500 CHN analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Soils with \leq 5% C (by mass) were defined as low C soils (Gill et al. 2023). Soil C and N were transformed to molar quantities to calculate soil C:N ratios. A portion of the frozen soil was thawed and sieved to 2 mm, and the pH was measured in a 1:2 volumetric ratio of soil and deionized water (Allen 1974). Soil moisture was measured as gravimetric water content (GWC), quantified by drying soils for 24 h at 105°C (Bradford et al. 2008).

2.4 | Soil Microbial C and N Cycling

Functional assessments of microbial communities were measured from thawed soils previously frozen at -20°C. Net N mineralization and nitrification rates were measured using a 28-day lab incubation (Robertson and Groffman 2015). Immediately after sieving to 2mm, 10g dry-equivalent soil was added to 50 mL of 2 M KCl and shaken vigorously by hand (Day 0) (Keiser, Knoepp, and Bradford 2016; Robertson et al. 1999). Another 10g dry-equivalent soil was incubated at 20°C in the dark for 28 days and checked weekly to maintain soil moisture at gravimetric moisture from field collection. At Day 28, the soil was extracted in 50mL of 2M KCl and shaken. Inorganic N concentrations (NH₄⁺-N and NO₃⁻-N) were quantified spectrophotometrically with a BioTek Synergy HTX Multimode Reader (Agilent, Santa Clara, CA, USA) using a modified salicylate assay and vanadium (III) assay, respectively (Hood-Nowotny et al. 2010). Net N mineralization rates were calculated as the difference in total inorganic N after 28 days, while nitrification rates were calculated as the difference in NO₃⁻-N. The nitrified fraction of mineralized N $(f_{\rm nitrified})$ was calculated by dividing net nitrification rate by net N mineralization rate.

We measured SIR as an estimate of active soil microbial biomass. SIR was measured after shaking 5g dry-equivalent soil with autolyzed yeast solution for 1 h at 100 rpm inside capped, 50 mL tubes with two replicates per sample (Anderson and Domsch 1978; Bradford, Fierer, and Reynolds 2008). After a 4h incubation, CO_2 -C in the headspace was quantified using a LI-7000 CO_3 /H₂O analyzer (LICOR, Lincoln, NE, USA).

Soil EEAs were measured using short-term, room-temperature assays with fluorometric methylumbelliferone (MUB) substrates in a modified universal buffer at a given soil's pH (German et al. 2011; Saiya-Cork, Sinsabaugh, and Zak 2002). Betaglucosidase (BG; EC 3.2.1.21) and N-acetylglucosaminidase (NAG; EC 3.2.1.14) activities were measured in 96-well plates with eight replicates for each enzyme per sample and included MUB curves, substrate controls, and soil homogenate controls. Prior to these assays, K_m tests for each site were performed to determine the times and substrate concentrations to achieve the maximum reaction rate (V_{max}) (Keiser et al. 2019). Fluorescence was measured at 360/450 nm (excitation/emission) with a BioTek Synergy HTX Multimode Reader. To evaluate microbial enzymatic investment for labile C and N, we calculated the ratio of BG and NAG activities, both natural-log transformed, with lower ratios indicative of higher N relative to C demand (Nieland et al. 2024; Sinsabaugh and Follstad Shah 2012).

2.5 | Statistical Analysis

All statistical analyses were done in R V4.3.3 (R Core Team 2024) using *tidyverse* to handle and visualize data (Wickham et al. 2019). We used linear mixed effects (LME) models and linear models for all analyses. To test for differences in background N deposition, we first used linear models to determine if mean annual background total N deposition and wet NH_4^+ deposition from 2013 to 2017 were different among the low, intermediate, and high deposition decreased from 2013 to 2017 by using LME models that included N deposition rank (low, intermediate, and high N deposition) and years as interacting fixed effects with sites being random effects to account for repeating measurements using packages *lme4* and *lmerTest* (Bates et al. 2015; Kuznetsova, Brockhoff, and Christensen 2017).

We used stepwise modeling to identify linear models that describe net nitrification rates and test H1. Following the Keiser, Knoepp, and Bradford (2016) and Gill et al. (2023) approach, we used model selection among known drivers of nitrification to isolate the best model that describes nitrification, first excluding and then including background N deposition. The first model selection exercise tested for the best model using predictors identified by Keiser, Knoepp, and Bradford (2016) and tested at a continental scale by Gill et al. (2023): net N mineralization rates, soil C, and GWC as interacting explanatory variables. Using Akaike information criterion (AIC) from the R package MASS to remove variables (Venables and Ripley 2002), the best-fit model included net N mineralization rate, soil C, soil moisture, and the interaction of N mineralization rate and soil moisture as predictive variables for net nitrification rates (adjusted $R^2 = 0.313$, p < 0.001, AIC = 193.6). The second model selection exercise included background (2013-2017) total N deposition as a variable, along with net N mineralization rates, soil C, and GWC (allowing them to interact), to describe net nitrification rates. Background total N deposition, rather than wet NH_4^+ , was used because it accounts for other deposited N species that can influence plant-microbe and microbe-microbe competition for N. According to AIC and analysis of variance (ANOVA), the model from the second exercise was a better fit than the model from the first exercise (AIC=188.0, p=0.013); thus, we report results from the second model.

Additional models were implemented to test H1. Because other soil characteristics can influence nitrification (Keiser, Knoepp, and Bradford 2016), we designed a separate linear model using the stepwise approach to test the effects of net N mineralization, soil moisture, soil pH, soil C, soil C:N, and their interactions on net nitrification rates, which were visualized using the interactions R package (Long 2019). Soil N was excluded due to its collinearity with soil C. Moreover, we used linear models to test if $f_{\text{nitrified}}$ was different between soils with low C and high C and among ecosystem types, including their interaction. We further tested H1 by assessing whether net nitrification and N mineralization rates and $f_{\text{nitrified}}$ were related to soil microbial functions. To do this, we used linear models of net nitrification, net N mineralization, and $f_{\rm nitrified}$ that separately tested active microbial biomass, log(NAG), and microbial enzymatic C:N investment as fixed effects interacting with N deposition rank.

To further test H1, we built a structural equation model (SEM) to determine how N deposition class (low, intermediate, and high) changed the effects of soil chemistry, particularly soil C, and microbial functions on $f_{\text{nitrified}}$. We first designed model paths a priori from existing literature (Figure S2; Table S2) and added linear models into an SEM using the piecewiseSEM package (Lefcheck 2016). We then checked the fit of the SEM using χ^2 and Fisher's C statistics which showed that the data fit poorly to the SEM ($\chi^2 = 25.37$, df = 6, p < 0.001; Fisher's C = 28.74, df = 12, p = 0.004). A d-separation test (Shipley 2013) indicated that adding a path between microbial enzymatic C:N investment and soil pH to the SEM would improve fit. In addition, a linear model predicting $f_{\text{nitrified}}$ from soil moisture was unnecessary based on its *p*-value (p = 0.555); we subsequently removed this linear model from the SEM. After making these changes, the overall fit of the SEM improved ($\chi^2 = 3.61$, df = 3, p = 0.307; Fisher's C = 5.12, df=6, p=0.529). We tested if N deposition class changed the magnitude of effects by performing a multigroup analysis using the multigroup function in *piecewiseSEM*. Standardized coefficients and p-values were gathered to compare outputs of each N deposition class, and we calculated the direct, indirect, and total effect of soil C on $f_{\text{nitrified}}$ among each N deposition class.

For H2, we used LME models to assess whether there were differences in net N nitrification and N mineralization rates between 2020 and 2021 for the resampled sites with individual plots as a random effect. These LME models included site, collection, and year as interacting fixed effects. We further tested H2 by using an LME model to test for differences in $f_{\text{nitrified}}$ between year and soil C as interacting fixed terms, with plot as a random effect.

To test if soil characteristics varied across sites, we used stepwise modeling to select the best-fitting linear model for the response variables soil pH, soil %C, soil %N, and soil C:N. Model predictors included climate (xeric or mesic), ecosystem (coniferous forest, deciduous forest, grassland, or residential yard), and deposition rank. We also modeled soil moisture as a function of these factors, excluding N deposition rank. Background N deposition was modeled as a categorical rather than continuous variable for ease of interpretation, and scrub and oak-palmetto forest ecosystems were excluded due to low replication. Two-way interactions among all predictor variables were included in the models. To meet model assumptions, some response variables were natural-log transformed or expressed using Yeo–Johnson transformation (Tables S3 and S4). ANOVA approximations were used to acquire *F*- and *p*-values for models, and significant effects or interactions were tested using Tukey's post hoc analysis in package *emmeans* (Lenth, 2023). Significance was set at $\alpha \leq 0.05$.

3 | Results

3.1 | N Deposition

Background total (wet + dry) N deposition rates (2013–2017) varied strongly across the contiguous United States, ranging over an order of magnitude among our 39 sites in 14 regions (Figure 2a). Most of the high background N deposition sites were in the Midwest (n=6; 9.8 ± 0.4 kg N ha⁻¹ year⁻¹), and all the sites in Northern Rockies and California (n=10; 4.7 ± 1.4 kg N ha⁻¹ year⁻¹) had low background N deposition. Background total N deposition rates increased significantly from the low to intermediate to high classes (all p < 0.001). Regardless of deposition class, however, total N deposition rates declined annually by $0.14 \text{ kg N ha}^{-1} \text{ year}^{-1}$ from 2013 to 2017 ($F_{1,153} = 10.9$, p = 0.001; Figure 2b) consistent with other studies (Ackerman, Millet, and Chen 2019; Benish et al. 2022). High N deposition sites had higher mean annual wet NH_4^+ deposition (2.9 ± 1.1 kg $NH_{4}^{+}-Nha^{-1}year^{-1}$) than low $(1.1 \pm 0.4 \text{ kg } NH_{4}^{+}-Nha^{-1}year^{-1})$ and intermediate $(1.7 \pm 0.5 \text{ kg } \text{NH}_{4}^{+}\text{-N} \text{ha}^{-1} \text{year}^{-1})$ sites (p < 0.001), but unlike total N deposition annual wet NH₄⁺ deposition rates did not decline from 2013 to 2017. While cumulative dry N deposition decreased significantly in 2019 from the 2013-2017 mean for three of the six CASTNET stations surveyed, it fell to its lowest values in 2020 for five stations (Figure S3). Cumulative dry N deposition returned to 2013-2017 ranges in 2021 (Figure S3).

3.2 | Net Nitrification and N Mineralization Rates

Low C soils (\leq 5% C by mass; Gill et al. 2023) had net nitrification rates that predominantly aligned 1:1 with net N mineralization rates, as indicated by $f_{\text{nitrified}} = 1$ (Figure 3a). Indeed, $f_{\text{nitrified}}$ was twofold greater in low C soils (0.945) than in high C soils (0.417; $F_{1.60}$ = 15.87, p < 0.001; Figure 3c) and was not different among coniferous and deciduous forests and grasslands (p > 0.05). Net nitrification rates were very low or undetectable in many of the high C soils, among which net N mineralization rates varied widely, indicating that the net N transformations were highly or entirely decoupled from one another in these soils. Yet, net nitrification and N mineralization rates did not always decouple at high soil C sites, with $f_{\text{nitrified}}$ varying between 0 and 1 (Figure 3a). The best-fit model from the model selection exercises predicting net nitrification rates included background mean N deposition as a covariate (t=-3.02, p=0.004; Figure 3b), along with net N mineralization (t=1.09, p<0.001), soil C (t=-2.31, p=0.024), and soil moisture



FIGURE 2 | Distribution of site locations and background total (wet + dry) N deposition. (a) Map of the contiguous United States showing the 14 regions and average background N deposition rates from 2013 to 2017. Shapes of the points correspond to background N deposition classification. (b) Interannual variability in background total N deposition rates from 2013 to 2017 across N deposition class ranks.

(t=-1.87, p=0.065; adjusted $R^2=0.379)$. Net nitrification rates were negatively related to soil C as expected (Table 1). Under low soil moisture content, soils with higher net N mineralization rates resulted in higher net nitrification rates (t=-3.32, p=0.001). In contrast, net nitrification rates increased with soil moisture at sites with higher mean background N deposition rates but decreased under lower background N deposition (t=2.93, p=0.005).

The model testing the effects of soil characteristics and net N mineralization explained 72% of the variation in net nitrification rates. In this model (Table 2), net N mineralization rates interacted positively with soil pH (t=2.86, p=0.006) and soil C (t=2.56, p=0.013) but negatively with soil C:N (t=-3.32, p=0.002) and moisture (t=-2.10, p=0.04). Net nitrification rates increased more with

higher net N mineralization rates under drier, lower soil moisture (Figure S4). However, at any given net N mineralization rate, soils with lower C:N ratios (i.e., more N relative to C) or higher pH had higher net nitrification rates (Figure S4).

3.3 | Soil Chemistry

Background N deposition classification was identified as a significant predictor for soil pH, total C and N, and soil C:N (Table 3); soil characteristics that also predicted net nitrification rates (Table 2). Except in the case of the soil C:N ratio, N deposition classification also interacted significantly with ecosystem type to explain soil chemistry variation (Table 3). Soil C and N concentrations



FIGURE 3 | Coupled-decoupled relationships between net N transformations as related to (a) soil C and (b) background N deposition rates. Coupled net N transformations align closely to a 1:1 relationship between net nitrification and N mineralization rates ($f_{nitrified} = 1$). Decoupled net N transformations fall along the horizontal lines ($f_{nitrified} = 0$). $f_{nitrified}$ values greater than 1 indicate net nitrification rates were greater than net N mineralization rates. (c) The $f_{nitrified}$ values across low and high soil C environments visualized as boxplots. In the boxplots, the interquartile range is shown as the total height of the box and the solid line within the box as the median, and the whiskers extend to either 1.5 times the interquartile range or the minimum or maximum values of the data. The number of independent observations is 75 (n = 75).

decreased across increasing N deposition classes for coniferous forests and grasslands by an average of 59% and 60%, respectively (Figure S5; all p < 0.05), but did not change for deciduous forests or yards. Collectively, soil C:N ratio declined from intermediate to high N deposition (p=0.013) by 2.2 units, with coniferous and deciduous forests having higher soil C:N ratios than grasslands and yards (Figure S5; all p < 0.001). While coniferous forest soil

pH decreased from 6.0 ± 0.4 in low N deposition sites to 4.7 ± 0.9 in intermediate N deposition sites (Figure S5; p=0.006), yard soil pH instead increased from 6.1 ± 1.1 in low N deposition sites to 7.1 ± 0.5 in high N deposition sites (Figure S5; $p\leq0.013$). In contrast to other soil properties, soil moisture was best explained by ecosystem type and time, reflecting that soils were wetter at the first collection than at the fourth collection ($F_{1,64}=47.02, p<0.001$)

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TABLE 1The best-fitting model explaining net nitrification ratesthat excludes soil pH and soil C:N.

Parameter	Estimate	t	р
Net N mineralization rate	1.091	5.41	< 0.001
Soil C	-0.120	-2.31	0.024
Soil moisture	-5.128	-1.87	0.065
Mean historical N deposition	-0.345	-3.02	0.004
Net N Mineralization Rate × Soil Moisture	-1.666	-3.32	0.001
Soil Moisture × Mean N Deposition	1.04	2.93	0.005

Note: Background rates of N deposition were included since the model fit improved with its inclusion (AIC, 188.0 < 193.6 when excluding N deposition). All fixed effects were modeled as continuous variables. Unstandardized coefficients are reported for estimates.

p-values less than 0.05 are bolded.

Adjusted $R^2 = 0.379$.

TABLE 2 |
 The best-fitting soil characteristics model explaining net nitrification rates.

Parameter	Estimate	t	D
Net N mineralization rate	0.241	0.38	0.703
Soil C	-0.040	-0.46	0.648
Soil moisture	5.873	3.12	0.003
Soil C:N	0.024	0.68	0.500
Soil pH	0.077	0.51	0.611
Net N Mineralization Rate × Soil C	0.155	2.56	0.013
Net N Mineralization Rate × Soil Moisture	-1.152	-2.10	0.040
Net N Mineralization Rate × Soil C:N	-0.075	-3.32	0.002
Net N Mineralization Rate × Soil pH	0.186	2.86	0.006
Soil C×Soil Moisture	-0.524	-2.34	0.023

Note: All fixed effects were modeled as continuous variables. Unstandardized coefficients are reported for estimates.

p-values < 0.05 are bolded.

Adjusted $R^2 = 0.719$.

and that deciduous forests had the highest soil moisture content at the first collection (all p < 0.05). Climate only emerged as a predictor for the soil pH, albeit insignificantly (Table 3).

3.4 | Soil Microbial Functions

Microbial enzymatic investment for C relative to N acquisition through the ratio of the C-acquiring enzyme BG with the

TABLE 3 I The best-fitting soil chemistry models.				
Response	Model	<i>F</i> , <i>p</i>		
Soil pH	~ Ecosystem + N dep + Climate + Ecosystem × N dep + Ecosystem × Climate	Ecosystem: $F_{3,57} = 30.12$, p < 0.001 N dep: $F_{2,57} = 3.41$, p = 0.040 Climate: $F_{1,57} = 2.95$, p = 0.091 Ecosystem × N dep: $F_{5,57} = 2.88$, p = 0.031 Ecosystem		
		× Climate: $F_{1,57} = 6.74, p = 0.012$		
Soil C ^a	~ Ecosystem + N dep + Ecosystem × N dep	Ecosystem: $F_{3,59} = 11.38$, p < 0.001 N dep: $F_{2,59} = 4.89$, p = 0.011 Ecosystem \times N dep: $F_{4,59} = 3.63$, p = 0.010		
Soil N ^a	~ Ecosystem + N dep + Ecosystem × N dep	Ecosystem: $F_{3,59}=3.73,$ p=0.016 N dep: $F_{2,59}=2.86,$ p=0.065 Ecosystem × N dep: $F_{4,59}=6.35,$ p<0.001		
Soil C:N ratio ^a	~ Ecosystem + N dep	Ecosystem: $F_{3,63} = 22.08$, p < 0.001 N dep: $F_{2,63} = 4.49$, p = 0.015		
Soil moisture	~ Ecosystem + Time + Ecosystem × Time	Ecosystem: $F_{3,64} = 9.63,$ p < 0.001 Time: $F_{1,64} = 47.02,$ p < 0.001 Ecosystem \times Time: $F_{3,64} = 3.29,$ p = 0.026		

Note: Ecosystem, N deposition (N dep), and climate fixed effects were all modeled as categorical variables.

Fixed effects with *p*-values < 0.05 are bolded.

^aSoil chemistry responses were log-transformed to meet normality assumptions.

N-acquiring enzyme NAG (BG:NAG ratio) can be used as a microbial N demand index (Nieland et al. 2024; Sinsabaugh and Follstad Shah 2012). Our results show that when $f_{\rm nitrified}$ was low, generally in high C soils, the ratio of BG:NAG was also low, indicating relatively high microbial N demand ($F_{1,68}$ = 23.42, p < 0.001; Figure 4a). The relationship between the BG:NAG ratio and net nitrification rates was also positive ($F_{1.68}$ = 12.10, p < 0.001; Figure 5a); in contrast, net

N mineralization rates decreased under higher BG:NAG ratios (i.e., lower net N mineralization rates with lower relative microbial N demand) ($F_{1,71}$ = 5.04, p = 0.028; Figure 5b). Significant interactive effects of background N deposition and NAG activity on net nitrification ($F_{2,71}$ = 3.21, p = 0.046) and N mineralization rates ($F_{2,71}$ = 4.12, p = 0.02) signal how background N deposition changed microbially mediated N cycling. Post hoc tests show that net nitrification and N mineralization



FIGURE 4 | Correlations between $f_{nitrified}$ and net N mineralization with functional assessments of soil microbial communities. $f_{nitrified}$ increased with (a) microbial enzymatic investment for C relative to N (BG:NAG ratio), an index of microbial N limitation (n = 74), but decreased with increasing (b) active microbial biomass (n = 78). The $f_{nitrified}$ correlations were not different among background N deposition classes. (c) The association between net N mineralization and active microbial biomass varied significantly with N deposition (n = 78). Net N mineralization rates correlated significantly with active microbial biomass at low N deposition ($\beta = 0.640$, p = 0.020), but not at intermediate ($\beta = 0.085$, p = 0.358) or high ($\beta = -0.2878$, p = 0.096) N deposition as indicated by the dotted linear regressions. Line colors correspond to the point colors reflecting background N classification.



FIGURE 5 | The relationship of (a) net nitrification and (b) net N mineralization rates and microbial enzymatic investment for C relative to N (microbial N limitation as indicated by the BG:NAG ratio, where higher BG:NAG indicates greater microbial investment in C relative to N acquisition). Each point is the net N transformation rate for each site at a collection time (n=74). The black lines show the correlations between the net N transformations and the microbial N limitation index.

rates increased with NAG activity at low N deposition but decreased at intermediate (p = 0.045) and high N deposition (p = 0.031), respectively (Figure S6).

Active microbial biomass was associated negatively with $f_{\text{nitrified}}$ ($F_{1,71}$ =4.71, p=0.033), such that a larger active microbial biomass

pool led to a smaller fraction of mineralized N that was nitrified (Figure 4b). However, active microbial biomass interacted significantly with N deposition for net N mineralization ($F_{2,71}$ =5.09, p=0.009), in that the relationship between active microbial biomass and net N mineralization rates switched from positive to negative as background N deposition increased (Figure 4c; p=0.012).

3.5 | SEM Analysis

The SEM revealed that the strength of the relationships among soil C, soil N, microbial biomass, microbial enzymatic C:N, and soil pH with $f_{\text{nitrified}}$ diminished as N deposition increased (Figure 6). Moving from low to high N deposition classification, the effect sizes of soil chemistry and microbial functions on $f_{\text{nitrified}}$ and its predictors generally declined and became insignificant (Figure 6a–c). The direct effect of soil C on $f_{\text{nitrified}}$ was significantly negative while N concentration effects were significantly positive at low N deposition (Figure 6a). However, soil C was not significantly associated with $f_{\text{nitrified}}$ at intermediate and high N deposition, with only soil pH being positively related to $f_{\text{nitrified}}$ at intermediate N deposition. Collectively, the total (direct + indirect) effect of soil C on $f_{\text{nitrified}}$ decreased with increasing N deposition classification (Figure 6d).

3.6 | Interannual Variability in Net N Transformations

Despite a decline in external N inputs in the year 2020 and an increase back to pre-2020 rates (2013–2017) in 2021 (Figure S3), there were no major differences in net N transformation rates between 2020 and 2021 (Figure 7). Only one of the seven sites (mixed forest—N CT) had higher net N mineralization rates in 2021 compared to 2020 (Figure 7a). A significant three-way interaction among site, time, and year for net nitrification rates ($F_{15,88}$ =1.85, p=0.040) indicated that rates were sometimes higher in 2021 than in 2020 for two sites, with no differences in

rates between 2020 and 2021 for the other five sites (Figure 7b). Four sites had little-to-no net nitrification in 2020 and 2021, resulting in $f_{\rm nitrified}$ values close to 0. In contrast, one site (oak-hickory—S CT) had 2020–2021 mean $f_{\rm nitrified}$ of 0.173 while two sites (suburban—PA and urban—PA) had $f_{\rm nitrified}$ values greater than 1. $f_{\rm nitrified}$ was greater in 2021 (0.06±0.11) than 2020 (0.02±0.04; $F_{1,84}$ =9.76, p=0.002) but only after excluding the two PA sites from the analysis. When including the PA region, however, $f_{\rm nitrified}$ decreased in 2021 (0.46±0.74) compared to 2020 (0.77±1.48) because of their greater overall rates compared to the remaining sites ($F_{1,115}$ =4.36, p=0.039). Soil C was an insignificant parameter in explaining net N transformations and $f_{\rm nitrified}$.

4 | Discussion

4.1 | Fraction of Mineralized N That is Nitrified Potentially Tied to Microbial Competition

Across an anthropogenic N deposition gradient within the contiguous United States, we tested whether N deposition weakened soil C control over the coupling between net nitrification and N mineralization rates when plants are excluded. We found that soil C was negatively related to net nitrification rates and resulted in $f_{\text{nitrified}}$ either close to 0 (decoupled N transformations) or 1 (coupled N transformations), supporting the hypothesis that soil C controls competition for N between heterotrophic microbes and nitrifiers (Dijkstra et al. 2008). A recent study leveraging Long-Term Ecological Research data across various



FIGURE 6 | Soil chemistry and microbial functional effects on $f_{\text{nitrified}}$ under (a) low, (b), intermediate, and (c) high N deposition. Paths in the SEM (χ^2 =3.61, df=3, p=0.307; Fisher's C=5.12, df=6, p=0.529) include the standardized effect sizes (boxes) under the different N deposition classes with solid lines indicating significant relationships at the *p<0.05, **p<0.01, and ***p<0.001 levels that are sized proportionally to the effect size for the 71 independent observations (n=71). Dotted lines show the insignificant paths for each deposition classification. The correlated error between soil C and N was 0.876. (d) The calculated direct, indirect, and total effects of soil C on $f_{\text{nitrified}}$ at low, intermediate, and high N deposition.



FIGURE 7 | Net N transformation rates in 2020 and 2021 collected at the resampled sites to assess temporal changes resulting from changes in N deposition from the COVID-19 pandemic (n=154). (a) Net N mineralization rates were higher in 2021 than in 2020 for the mixed forest—S. CT site. (b) Net nitrification rates varied significantly between 2020 and 2021 for some collection points for two sites. Points are the mean rates (± 1 standard error) at each time point separated by year. Asterisks above points in (b) indicate statistical differences between years at the time of collection at the **p < 0.01 and ***p < 0.001 thresholds.

biomes and climates in North America also documented that soil C influenced the degree of coupling of net N transformations across ecosystems (Gill et al. 2023). Labile C availability primarily regulates how much N is released by microbial heterotrophs (Keiser, Knoepp, and Bradford 2016). Although we did not quantify labile C availability, this pool correlates positively to the measured total soil C (McLauchlan and Hobbie 2004). Moreover, higher soil C:N ratios resulted in much lower net nitrification rates measured using laboratory incubations even under relatively high net N mineralization rates. This finding of low net nitrification rates under high soil C:N environments could be more pronounced in the presence of roots because plants also compete with nitrifiers for NH₄⁺, further restricting nitrification (Schimel and Bennett 2004). Thus, our analysis across multiple terrestrial ecosystems that vary in soil characteristics and climates offers evidence that soil C availability drives coupled-decoupled net N transformations whereby $f_{\rm nitrified}$ associates negatively with increasing soil C.

Although we did not explicitly measure competition for NH₄⁺ in this study using gross rates, our functional assays suggest that competition for N between microbial heterotrophs and nitrifiers appeared to shape soil N dynamics. Soil microorganisms synthesize fewer extracellular enzymes that target SOM for labile N (i.e., NAG) under higher available N conditions as a mechanism to conserve intracellular resources (Allison and Vitousek 2005; Chróst 1991; Nieland et al. 2024; Sinsabaugh et al. 2008; Sinsabaugh and Follstad Shah 2012). Microbial enzymatic investment for C relative to N acquisition, the BG:NAG ratio, serves as an indicator of microbial N limitation because it reflects the balance between bioavailable N, largely controlled by plant N uptake and heterotrophic N requirements (Fierer, Wood, and Bueno De Mesquita 2021; Sinsabaugh, Hill, and Follstad Shah 2009), despite NAG being a C- and N-acquisition enzyme. Lower microbial heterotrophic N demand has been associated with higher net nitrification and mineralization rates (Jia et al. 2020; Jian et al. 2016; Ouyang et al. 2018; Vega Anguiano et al. 2024), and we found some evidence that $f_{\text{nitrified}}$ increased also as microbial N demand decreased (Figure 4a). However, the SEM indicated that microbial N demand was weakly associated with $f_{\text{nitrified}}$ when other pathways in explaining $f_{\text{nitrified}}$ were also included. This contrast between the SEM and the linear model highlights that other relationships tied to $f_{\rm nitrified}$ besides microbial N demand measured using extracellular enzymes are necessary to explain how microbial competition for N affects $f_{\text{nitrified}}$.

The negative relationship between active microbial biomass and $f_{\rm nitrified}$ further supports the idea that competition between microbial heterotrophs and nitrifiers may drive the relationship between soil C and net N transformations. Soil microbial heterotrophs compete with nitrifiers for NH⁺ (Verhagen, Laanbroek, and Woldendrop 1995), with gross immobilization rates exceeding gross nitrification rates in some cases (Hart et al. 1994). A recent synthesis found that soil microbial biomass C, quantified through chloroform fumigation extraction, is a driver of gross N immobilization rates (Li et al. 2021). Hence, greater soil microbial biomass, particularly the active pool measured through SIR, should increase N immobilization and limit nitrification (Li et al. 2020; Schimel and Bennett 2004). Ectomycorrhizal (EcM) fungi may also play a key role in $f_{\rm nitrified}$ in soils as these organisms are known to compete with nitrifiers for NH4+ (Tatsumi et al. 2020) and associate with trees at some of our sites (Table S1; Phillips, Brzostek, and Midgley 2013). Given that net N mineralization and nitrification rates were measured in the laboratory, bioavailable N may be greater than would be expected in the presence of roots and their mycorrhizal symbionts. Altogether, our functional assessments of soil microbial communities provide further evidence that the mechanism for net nitrification and N mineralization coupling is explained by microbial competition for NH_4^+ .

4.2 | N Deposition Modifies Net N Transformation Dynamics

Background rates of N deposition partially explained net nitrification rates and $f_{\text{nitrified}}$, with $f_{\text{nitrified}}$ increasing under high background N deposition in high soil C, supporting H1 that N deposition alleviates NH₄⁺ limitation of nitrifiers (Figure 1). Previous research found that soil C was the primary driver determining the degree of coupling of net N mineralization and nitrification in terrestrial landscapes (Gill et al. 2023; Keiser, Knoepp, and Bradford 2016). Our analysis suggests that background N deposition explained $f_{\text{nitrified}}$ in addition to soil C. Many of the high soil C sites that deviated from their predicted decoupled net nitrification-N mineralization relationship (i.e., $f_{\text{nitrified}} = 0$) had intermediate or high background N deposition. Moreover, the total effect of soil C on $f_{\text{nitrified}}$ diminished as background N deposition increased. These findings could explain why some high soil C ecosystems reported by Gill et al. (2023) had coupled net N transformations, particularly for the Midwest and Atlantic coast sites where dry deposition of ammonia (NH₂) is high because of agriculture (Liu et al. 2022). Furthermore, these Midwest and Atlantic coast soils were relatively enriched with N based on soil C:N ratios (Figure S3); thus, net nitrification rates and $\boldsymbol{f}_{\rm nitrified}$ should increase with more available N (Elrys et al. 2021). Because wet and dry deposition has been NH₄⁺ dominated in the United States in recent years (Li et al. 2016), N deposition should continue to alleviate NH_4^+ limitation for nitrifiers, with oxidized forms of deposited N supplying N to plants and microbial heterotrophs, weakening competition with nitrifiers.

Site-specific characteristics may partially explain net nitrification rates. For example, our analysis showed that under drier soil conditions, high net N mineralization rates resulted in high net nitrification rates. Conversely, soils with high moisture content and high background mean N deposition supported high net nitrification rates even when net N mineralization rates were low. The source of available NH₄⁺ for nitrifiers may therefore switch from N mineralization to deposition, and vice versa, under changing soil moisture conditions that could reflect site-specific edaphic characteristics, such as soil texture. However, our inferences on soil moisture and nitrification rates are limited since soil moisture was excluded from the SEM and given that laboratory incubations were run under field moisture conditions rather than at 65% water holding capacity (Linn and Doran 1984). Another soil characteristic, soil pH, also interacted with net N mineralization rates to explain net nitrification rates. Nitrification activity is generally more favored in neutral soils since NH₂ availability, the substrate for ammonia oxidizers, declines at lower pH conditions due to NH₄⁺ ionization (Frijlink et al. 1992). While site-specific soil characteristics influence soil microbial activity (Zeglin et al. 2007) and N access (Keiser, Knoepp, and Bradford 2016) and can explain some variation in nitrification rates across regions, our analysis reveals the dual control of soil C and background N deposition as potential largescale drivers of $f_{\text{nitrified}}$.

The functional assessments of enzyme activity and active microbial biomass that indicate probable soil microbial competition for N when considered together changed in response to increasing background N deposition. At low N deposition, active

microbial biomass and net N transformation rates were positively related as expected (García-Ruiz et al. 2008; Hobbie 2015), with the SEM confirming the negative association with active microbial biomass and $f_{\text{nitrified}}$. However, at intermediate and high N deposition, active microbial biomass and NAG activity correlations with net N transformations unexpectedly turned negative, as well as active microbial biomass insignificantly relating to $f_{\text{nitrified}}$ in the SEM. A recent meta-analysis reports NAG activity is suppressed at N application rates at or exceeding 83 kg N ha⁻¹ year⁻¹ (Jia et al. 2020), but our results show NAG activity decreased at the high N deposition level, that is, at N input rates about an order of magnitude lower than that published threshold. This finding indicates that soil microbial communities are sensitive to external N supplied at rates much lower than those typically applied in N-fertilization studies (Averill, Dietze, and Bhatnagar 2018), suggesting a low critical N load to alter soil C-N dynamics. Although the reason for this large difference in thresholds is not known, plants and soil microbes take up a small fraction of applied fertilizer N because their net sink rates are likely saturated (Lovett and Goodale 2011). In contrast, in most regions of the world, N deposition rates are lower than agricultural fertilization rates and consistent over time, allowing for N accumulation in ecosystems. Future work should discern the quantity of anthropogenic N required to alter competition for N between microbial heterotrophs and nitrifiers and explicitly test microbial competition with measures of gross N transformation rates across ecosystems.

4.3 | Spatiotemporal Dynamics of Microbial Competition for N

Despite a reduction in N deposition related to COVID-19 restrictions, there were no major differences in net N transformation rates between 2020 and 2021. Soil C did not control net N transformation rates nor $f_{\text{nitrified}}$ in 2021 at sites with intermediate and high background rates of N deposition, in contrast to H2 (Figure 1). Instead, it appears that background N deposition was a mediator of the N cycle. Our findings are supported by the few N-cessation field experiments that document soil net N mineralization (Clark et al. 2009; O'Sullivan et al. 2011) and nitrification rates (Stienstra, Klein Gunnewiek, and Laanbroek 1994) in previously fertilized treatments remaining higher than rates in unfertilized treatments for at least 10 years. These findings, along with our results, are evidence of microbial functional legacies in which contemporary microbial functions are driven by previous environmental conditions (Crowther et al. 2019; Hawkes, Shinada, and Kivlin 2020; Hawkes and Keitt 2015), or that high levels of N supply persisted. With higher N availability from past N deposition, the cumulative amount of anthropogenic N, rather than annually supplied N concentration, appears to mutually control $f_{\text{nitrified}}$ along with soil C. Therefore, a short-term dip in N deposition does not decrease net N transformation rates and $f_{\text{nitrified}}$. Consequently, soil microbial functions may not change in tandem with ongoing declines in N availability across most unmanaged landscapes (Mason et al. 2022; McLauchlan et al. 2017).

If background N deposition modifies the influence of soil C on net N transformations, legacies of elevated net N transformation rates and $f_{\text{nitrified}}$ could persist after a decrease in anthropogenic N deposition. The recovery of ecosystem pools and processes from high N availability likely occurs nonlinearly and asynchronously (Gilliam et al. 2019) because plants and soil microbes jointly drive this recovery (Nieland and Zeglin 2024). For example, low C:N ratio in SOM can support high rates of N mineralization after N fertilization ceases (Frankenberger and Abdelmagid 1985; Manzoni et al. 2008), but lower NH_4^+ availability due to immobilization can reduce nitrification rates and $f_{\text{nitrified}}$ within 5 years after N fertilization stops (Nieland and Zeglin 2024). Given that dry N deposition rates decreased across 2018-2020, it appears that 3 years of total dry N deposition decline was not long enough to disrupt the role of background N deposition across North American ecosystems. Plants also retain high concentrations of N in their biomass that later serve as a substrate for N once plant litter turns over (Cotrufo et al. 2015; Lavallee, Soong, and Cotrufo 2020). For example, over longer time scales, early-seral N2-fixing trees can leave legacies of elevated soil N availability that persist for tens to hundreds of years (Perakis, Sinkhorn, and Compton 2011; Von Holle et al. 2013). Thus, the effects of high background N deposition on soil C, net N mineralization, and net nitrification relationships may depend on the magnitude and duration of N deposition in combination with ecosystem-specific characteristics, such as plant community composition.

5 | Conclusions and Implications

Our measurements of net N transformations, $\boldsymbol{f}_{\rm nitrified}\text{,}$ and microbial biomass and function at 39 sites in 14 regions across the contiguous United States document that N deposition can disrupt the role of soil C as a gatekeeper of coupled net N transformations. As deposition supplied more N to soil pools, the relationship between net N mineralization and net nitrification rates $(f_{\text{nitrified}})$ weakened because heterotrophs and nitrifiers shifted their N source. However, background N deposition rates of previous years, instead of N deposition inputs during the years of observation, controlled soil microbial responses. This finding suggests previous N deposition has a stronger role in the contemporary N cycle than current deposition. While inherent ecosystem properties such as differences in vegetation or soil characteristics affect how ecosystems respond to higher N availability, our findings suggest that background N deposition uniformly disrupts the relationship between soil C and net N transformations across different ecosystems and climates. The cumulative effect of N deposition attenuates how soil C controls coupled net N transformations by decoupling NH_4^+ availability from soil N mineralization, which persists even with short-term (1-2 year) dips in deposition. With N deposition generally declining across the United States and more widely across the globe, it is unknown how long the historical imprint of N deposition will alter the relationship between soil C and net N transformations and whether this legacy will vary across soil and ecosystem types. In the short term, predictions of N transformations, N availability, and N losses should account for both soil C content and regional N deposition.

Author Contributions

Matthew A. Nieland: data curation, formal analysis, visualization, writing – original draft. Piper Lacy: data curation, writing – review and editing. Steven D. Allison: resources, writing – review and editing.

Jennifer M. Bhatnagar: resources, writing - review and editing. Danica A. Doroski: resources, writing - review and editing. Serita D. Frey: resources, writing - review and editing. Kristen Greaney: resources, writing - review and editing. Sarah E. Hobbie: resources, writing - review and editing. Sara E. Kuebbing: resources, writing review and editing. David B. Lewis: resources, writing - review and editing. Marshall D. McDaniel: resources, writing - review and editing. Steven S. Perakis: resources, writing - review and editing. Steve M. Raciti: resources, writing - review and editing. Alanna N. Shaw: resources, writing - review and editing. Christine D. Sprunger: resources, writing - review and editing. Michael S. Strickland: resources, writing - review and editing. Pamela H. Templer: resources, writing - review and editing. Corinne Vietorisz: resources, writing - review and editing. Elisabeth B. Ward: resources, writing - review and editing. Ashley D. Keiser: conceptualization, methodology, project administration, resources, supervision, validation, writing - review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.g79cnp611. Atmospheric nitrogen deposition data are available from the National Trends Network (National Atmospheric Deposition Program) at https://nadp.slh.wisc. edu/committees/tdep/ (version 2023.1) and from the Clean Air Status and Trends Network (United States Environmental Protection Agency) at https://www.epa.gov/castnet. Climate and weather data are available from the Cooperative Observer Network (National Weather Service) at https://www.ncdc.noaa.gov/cdo-web/datasets (daily summaries).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.