LAND-USE DISTURBANCE INCREASES SOIL NITROGEN TRANSFORMATION RATES BY ALTERING TRAJECTORIES OF FOREST RECOVERY

by

JESSIE IRIS MOTES

(Under the Direction of Nina Wurzburger)

ABSTRACT

Land-use disturbance alters the nitrogen (N) cycle in ecosystems, yet the mechanisms that lead to long-term changes to the N cycle are unclear. We investigated how land-use disturbance alters the long-term N cycle of a forest ecosystem through its effect on symbiotic nitrogen fixation (SNF) and mycorrhizal tree dominance. We found that increasing disturbance intensity led to increased SNF and, subsequently, increased arbuscular-mycorrhizal tree dominance. In turn, we found that arbuscular-mycorrhizal dominance indirectly led to increased N transformation rates, N pools, and the abundance of N cycling microbial genes by increasing soil pH. Our study presents a mechanism through which ecosystem processes are altered long-term and shows that N-fixing trees have the potential to create biogeochemical founder effects that influence trajectories of ecosystem recovery for decades.

INDEX WORDS: Land-use disturbance, nitrogen cycle, mycorrhizae, microbial ecology, soil chemistry

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DEDICATION

This thesis is dedicated to mom, dad, and Talley.

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CHAPTER 1

INTRODUCTION

Land-use disturbance has altered much of the terrestrial biosphere (Hurtt et al., 2006; Perring et al., 2016; Vitousek, 1997), making it critical to understand its effects on ecosystems. Land-use can have immediate and dramatic effects on the terrestrial nitrogen (N) cycle. For example, timber harvesting or land clearing removes N stocks, which can induce N limitation on forest ecosystem recovery (Swank et al., 2014; Webster et al., 2016). In contrast, agricultural activities can result in additions of N, that promote losses of reactive N (G. P. Robertson & Vitousek, 2009). However, it is less clear how land-use might affect the long-term cycling of N, and whether disturbance effects can persist for decades or centuries.

One way that land use disturbance can affect the N cycle is by promoting N-fixing tree species (i.e., trees capable of symbiotic nitrogen fixation (SNF)) (Boring et al., 1988; Zheng et al., 2020; Boring et al., 2014). N fixers can increase inorganic N availability (Montagnini et al., 1986), rates of mineralization and nitrification (Montagnini et al., 1986) as well as losses of reactive N (Kou-Giesbrecht & Menge, 2019). Therefore, N fixation is generally considered an ecosystem recovery mechanism because it can replenish N lost from disturbance (Boring & Swank, 1984; Chapman, 1935; Lu & Hedin, 2019). While poorly studied, N fixers may have legacy effects on soil N transformations (Von Holle et al., 2013), even though they tend to be outcompeted by non-fixers in later succession. This leads us to the question—do N fixers have lasting effects on soil N cycling in ecosystems recovering from prior land-use?

N fixers could have long-term effects on the N cycle if they cause biogeochemical founder effects—soil conditions that favor the subsequent establishment of certain trees (Lu & Hedin, 2019). N fixer founder effects might be particularly relevant to temperate forests, where arbuscular-mycorrhizal (AM) and ectomycorrhizal (ECM)-associated trees can both dominate. These two mycorrhizal types promote, and are favored by, distinct N cycling regimes, such that AM tree species promote an inorganic nutrient economy and ECM tree species promote an organic nutrient economy (Lu & Hedin, 2019; Phillips et al., 2013). As a result, AM and ECM trees have the potential to promote the long-term stability of N cycling, particularly if they represent alternative stable states (Lu & Hedin, 2019). Thus, it is possible that N fixers responding to land use disturbance promote a high availability of N, due to inputs of N through fixation. This inorganic nutrient economy may persist even after the N fixer has declined (Von Holle et al., 2013), promoting the dominance of AM over ECM trees in late succession. Thus, land use disturbance may trigger a transition from ECM to AM dominance and stabilize inorganic N cycling in the ecosystem for decades.

While AM and ECM-associated tree species promote divergent N cycles, the chemical and biotic mechanisms involved are unclear. One potential mechanism through which mycorrhizal dominance could alter the N cycle is through soil acid-base chemistry. For example, ECM dominated landscapes tend to have a lower pH than landscapes dominated by AM associated species (G. Lin, Craig, et al., 2022; G. Lin, Yuan, et al., 2022). ECM tree species have soil acidifying capabilities through their organic acid root exudates and leaf litter quality (Yin et al., 2014; Nilsson et al., 1982; Schrijver et al., 2012). Additionally, leaf litter with a slower decay rate, such as the litter produced by ECM tree species, tends to have a slower return rate of base cations to the soil which reduces the acid buffering capability (Deano & Robinson, 1985; Hobbie

et al., 2006; Keller & Phillips, 2019). Therefore, differences in soil pH caused by the presence of AM and ECM tree species could alter nutrient cycling and the bioavailability of nutrients – most macronutrients are bioavailable within a pH range of 6.5-8 (Aciego Pietri & Brookes, 2008; Kemmitt et al., 2006; McCauley et al., 2009).

In addition to acid-base chemistry, mycorrhizal dominance has the potential to alter the cycling of N through the composition of soil microbial communities. Plant species influence microbial community composition, which is a direct link between aboveground and belowground ecosystem processes (Westover et al., 1997). One specific way plants influence microbial composition is via root exudates and litter quality (Berg & Smalla, 2009; Aneja et al., 2006). Since AM trees tend to have litter with a lower C:N ratio compared to litter from ECM tree species (Phillips et al., 2013), these differences have the potential to create distinct microbial communities. Bacteria drive most inorganic N cycling whereas fungi are often responsible for the decomposition of organic complexes thus the relative dominance of each determines the prevalence of their soil processes (Boer et al., 2005). This idea is supported by soil microbiome analyses that show bacteria are generally more abundant in AM-dominated ecosystems, while fungi are more abundant in ECM-dominated ecosystems (Bahram et al., 2020; Cheeke et al., 2017), yet fungal diversity still remains greater in AM dominated stands (Eagar et al., 2022). Thus, mycorrhizal effects on microbial communities may be responsible for the different N cycle transformation rates, namely nitrification and denitrification, which are facilitated by microbes. Specifically, nitrification is a one- or two- step process where complete ammonia-oxidizing bacteria (CAOB) oxidize ammonia to nitrate (Daims et al., 2015; van Kessel et al., 2015) or ammonia-oxidizing archaea (Könneke et al., 2005) and bacteria (Kowalchuk & Stephen, 2001) (AOA and AOB) oxidize ammonia to nitrite, followed by a conversion to nitrate by nitrite-

oxidizing bacteria. Following nitrification, the stepwise reduction of nitrate to dinitrogen is performed by bacteria with the genes *nirK*, *nirS*, and *nosZ* (Throbäck et al., 2004). However, it is unclear whether mycorrhizal dominance alters the abundance of these specific bacteria and archaea responsible for N cycle transformations. One recent study did not find differences in the abundance of N cycling genes in AM and ECM-dominated forests (Saifuddin et al., 2021), but this question has not been explored in many forest ecosystems.

Prior work has demonstrated that forests with prior land-use disturbance tend to cycle N rapidly (Davidson & Swank, 1987; Keiser et al., 2016), and have different abundances of N cycling genes in soils (Osburn et al., 2021) compared to undisturbed forests. However, it has remained unclear how land-use disturbance triggers these changes in the N cycle and whether they are predictable. Here, we investigated the mechanism by which land use disturbance alters the N cycle, using a forest ecosystem with a dominant N fixer, and where AM and ECM trees appear to represent alternative stable states. We hypothesized that: 1) increasing land-use disturbance intensity promotes the abundance of an N fixer, and hence, rates of SNF, 2) these higher rates of SNF, would then facilitate the dominance of AM trees, and 3) AM dominance would be associated with rapid rates of soil N transformations, larger N pools, and the greater abundances of N cycling genes. To address these hypotheses, we coupled long-term forest composition data with new measures of soil N transformation rates, N pools and gene abundances. We developed structural equation models to test the relationship between disturbance-induced SNF, AM dominance, soil chemical variables (pH, N pools, and soil N transformations), and microbial gene abundances (*amoA, nirK, nirS*, and *nosZ* genes). Southern Appalachian forests provide a model ecosystem for testing these hypotheses because historical land-use practices (i.e., selective cutting, clear cutting, and agricultural abandonment) provide a

gradient in disturbance intensity, and black locust (*Robinia pseudoacacia* L.), a common early-successional tree species, with known effects on soil N transformations (Boring & Swank, 1984; Knoepp et al., 2008).

CHAPTER 2

METHODS

Site selection

Our research was conducted at the Coweeta Hydrologic Lab, a USDA Forest Service Experimental Forest, in western North Carolina, USA that is comprised of sub-watersheds with a range of disturbance treatments. The Coweeta basin holds 2185 ha of mixed-deciduous forest, that range in elevation from 675 m to 1592 m (Swank & Crossley, 1988). The mean annual temperature is 13°C and the mean annual precipitation is 1800 mm (Laseter et al., 2012). The soils range from immature inceptisols to older ultisols (Swank & Crossley, 1988).

We selected long-term vegetation plots across a range in aspect and elevation from three land-use disturbances. The first disturbance was selective-cutting, where plots experienced, on average, 30% basal area extraction which included the majority of trees over 15 inches at the stump, with the mean year of harvesting in 1921 (Douglass & Hoover, 1988). All skidding in the selectively-cut watersheds was done by horses and concluded in 1923. The selectively-cut watersheds were 2 and 14, which are 12 and 61 ha, respectively. The decline of the American chestnut (*Castanea dentata* (Marshall) Borkh.), which made up approximately 30% of the basal area at that time (Elliott et al., 1998), impacted the entire Coweeta basin. The decline began in 1933, and by 1940 all American chestnuts over 10 cm dbh were killed (Douglass & Hoover, 1988). The second disturbance was a 59 ha, previously mix hardwood watershed (watershed 7) that was clear-cut and cable-logged in 1977 using a cable system that kept logs entirely suspended above the ground (Swank et al., 2014). The third disturbance was a 9 ha watershed

(watershed 6) that underwent transition to agriculture and was subsequently abandoned in 1967. In 1941, 5 m of woody vegetation was cut from either side of the stream. In 1958, the watershed was clearcut and merchantable timber was removed while remaining debris was piled and burned. In 1959, the watershed was scarified, planted with fescue grass (*Festuca octiflora* Walter), limed, and then fertilized with a fertilizer comprised of N, phosphorus, and potassium. The fertilizer was applied again in 1965. In 1966 and 1967, the grass was herbicided then the watershed reverted to forest (Elliott et al., 1998; Swank & Crossley, 1988).

The clear-cut watershed and one of the selectively-cut watersheds are south-south east facing, and the agriculture abandonment watershed and the other selectively cut watershed are northwest facing. To better isolate effects of land-use and subsequent patterns in forest recovery from those determined by hillslope hydrology and soils, we selected 13-15 plots from each disturbance regime distributed across three hillslope positions: ridge, mid-slope, and toe-slope where possible for a total of 43 long-term vegetation plots across Coweeta. In the selectively-cut watersheds, plots were 800 m^2, in the clear-cut watershed, each plot contained two 25 m^2 subplots, and in the agriculture abandonment watershed plots were 200 m^2.

Symbiotic N fixation

We estimated cumulative SNF over time since disturbance by applying a framework for scaling SNF from individual trees to the long-term forest data. The number of forest surveys and their timing since disturbance varied by the type of land-use disturbance. Following the selective-cut disturbance (midpoint year 1921), forest plots were surveyed 4 times (1934, 1970, 1990, and 2010). For the clear-cut disturbance (1977), vegetation plots were surveyed five times (1979, 1984, 1993, 1997 and 2008). And following agricultural abandonment in 1968, vegetation plots were surveyed three times (1982, 1995 and 2012). Using these values of black locust stem

density from long-term data, we estimated annual black locust stem densities in each plot following disturbance by fitting linear mixed-effects models to stem density (square-root transformed) as a function of years since disturbance (natural-log transformed) with plot as a random effect. We then extracted coefficients from each plot and applied these predictions of stem density per year to a Monte Carlo simulation model that scaled fixation rates from individual trees to plots over time (Wurzburger et al., 2021). The simulation model was parameterized by previously established relationships between nodule presence, nodule biomass and activity, and forest age. Fixation rates of individual trees were aggregated for each plot and summed over time since disturbance to determine cumulative N fixation (kg N ha^{-1}).

Forest mycorrhizal dominance

We calculated the proportion of total stem basal area per plot that associates with AM fungi from forest survey data from the most recent survey year (selective-cut plots: 2010; clear-cut plots: 2008; agricultural abandonment plots: 2012). Dominant AM species in our plots were maple (*Acer rubrum* L.) and tulip poplar (*Liriodendron tulipifera* L.), and the dominant ECM species were red and white oak (*Quercus rubra* L. and *alba* L.). Some plots also had mountain laurel (*Kalmia latifolia* L.) and rhododendron (*Rhododendron maximum* L.) in the understory, which associate with ericoid mycorrhizal fungi.

Soil sampling

We removed the O horizon and sampled the top 10 cm of mineral soil in a stratified haphazard pattern from each plot during 2019 and 2020 from June through September. We collected 12 samples per plot from the selectively cut watershed, 10 samples (5 from each subplot) in the clear-cut watershed, and 9 from the agriculture abandonment watershed, where the number of samples scaled to the area of the plots. Prior to all analyses, soils from each plot

were sieved (2 mm) and homogenized, a subsample of field-fresh soil was frozen for gene abundance analysis at -60°C and the remaining sample was stored at 4°C for soil chemical analyses.

Soil chemistry

We measured soil pH using methods described by Robertson (1999). We weighed duplicate 15 g samples of sieved field-fresh soil into 100 mL acid-washed extraction cups. We added 30 mL of deionized water, mixed thoroughly, and allowed the mixture to stand for 30 minutes. We then mixed the solution immediately before measuring pH using a table-top pH meter. We dried a different subset of soils at 60° C for 72 hours, ground soils in a ball mill grinder to a fine powder and quantified percent C and N using the Micro-Dumas combustion analysis. We converted percent C and N to total C and N using previously published bulk density values for each watershed (0.695 g cm^{-3} for WS6 (Montagnini et al., 1989), 0.75 g cm^{-3} for WS7 (Boring & Swank, 1984), 0.55 g cm^{-3} for WS2 (Keiser et al., 2016), and 0.64 g cm^{-3} for WS14 (Montagnini et al., 1989).

We quantified total ammonium and nitrate pools by extracting 10 g of field-fresh soils in 100 mL of 2M KCl. We agitated the soil-KCl solution 8 times over 3 hours then allowed the soil to settle until the supernatant was clear. We decanted the clear supernatant through a Whatman #41 filter. We then filtered the solution through a 1µm syringe filter. The samples were frozen until colorimetric analysis using a continuous flow autoanalyser (Autoanalyzer 301, Alpkem Corporation, Clackamas, OR, USA), and ammonium and nitrate were expressed per gram of dried soil.

We measured the in-situ availability of ammonium and nitrate by burying mixed-bed resin bags at 10 cm mineral soil depth for 30 days using methods modified from Sparks et al.

(2020). We constructed the 5.5 x 6 cm resin bags using nylon material, sewn with polyester thread. We added four grams of 20-50 mesh ion exchange resin from BIO-RAD (AG® 501-X8 hydrogen and hydroxide form). We activated the resin bags by shaking fully submerged bags at 150 rpm on a reciprocating shaker for 10 minutes in 5 successive 0.5 M $NaHCO_3$. We rinsed the bags in DI water between each $NaHCO_3$ equilibration and stored our resins in DI water until they were used. We combined the bags from each plot and extracted the resin bags in 40 mL of 1M KCl per bag by shaking them at 120 rpm for two hours. The samples were frozen until we measured resin-extractable ammonium and nitrate with colorimetric analysis using a continuous flow autoanalyser (Autoanalyzer 301, Alpkem Corporation, Clackamas, OR, USA). We converted the concentration of ammonium and nitrate to μg N g $resin^{-1}$ day^{-1} based on the 30 days and the dry mass of the resin beads.

Soil N transformations

We measured potential denitrification using the acetylene block method on soils sampled in 2019 using modified methods from Baas et al., 2017 and Groffmann et al., 1999. We homogenized the soil samples, sieved them in a 2 mm sieve and stored them at 4° C for less than 24 hours. We weighed out 33.7 g of sieved, field-moist soils into an N-purged 250 mL chamber and added 33.7 mL of 1mM dextrose and 1 mM sodium nitrate solution. In order to make soils anaerobic, we performed three rounds of vacuum pumping the chamber to 20 in of Hg followed by injecting 120 mL of N_2. 25 mL of headspace was removed and replaced with 25 mL of acetylene. We then incubated the samples at room temperature while shaking on a reciprocating shaker at 125 rpm for 3 hours. We took 25 mL of headspace from the chambers at 30, 90, and 180 minutes. We ran triplicate assays per plot. We analyzed the change in N_2O concentration using the electron capture detector of an SRI 8610C GC (SRI Instruments, CA, USA).

We quantified potential mineralization and nitrification rates on soils sampled in 2019 using methods described by Robertson (1999). For the initial measurement, we used 10 g of field-fresh soil for each plot and extracted inorganic N with 2M KCl. In the lab, we weighed out triplicates of 10 g of field-moist soil from each plot and stored them in a 25° C incubator for 28 days. We maintained soil moisture at 55% by mass. We then extracted inorganic N from the soils with 2M KCl. The samples were frozen until we measured ammonium and nitrate concentration using colorimetric analysis using a continuous flow autoanalyser (Autoanalyzer 301, Alpkem Corporation, Clackamas, OR, USA). We converted the ammonium and nitrate concentration to potential mineralization and nitrification rates using formulas described in Robertson (1999).

Microbial gene abundance

We quantified microbial functional genes involved in nitrification and denitrification using qPCR. For nitrification, we quantified *amoA* genes from ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and complete ammonia-oxidizing bacteria (i.e., comammox, CAOB). We extracted DNA using a Qiagen Powersoil Kit from 250 mg of soils sampled from 2019 that had been stored at -60°C. We distributed samples from each disturbance type across two plates, and we used plate as a mixed effect in our models. For AOB qPCR, we used the primer pair amoA-1f/2r (Rotthauwe et al., 1997a), for AOA we used the primer pair Arch-amoAF/R (Francis et al., 2005), and for CAOB we used the primer pair comamoa F/R (Zhao et al., 2019). For denitrification, we quantified two nitrite reductase genes (*nirS, nirK*), and one nitrous oxide reductase genes (*nosZ*). We chose these genes because they represent two critical steps in the denitrification pathway: nitrite reduction (i.e., conversion of NO_2^- to NO) represents initial conversion of N to gaseous form, while nitrous oxide reduction (i.e., conversion of N_2O to N_2) is the terminal step of the pathway and prevents emission of N_2O, a potent

greenhouse gas. For *nirS*, we used the primer pair nirScd3af/nirSR3cd (Throbäck et al., 2004), for *nirK* we used the primer pair nirK876/nirK1040 (Henry et al., 2004), while for *nosZ* we used the primer pair nosZ2F/R (Henry et al., 2006). All qPCR reactions contained 10 µl Quantitect SYBR master mix (Qiagen, Valencia, CA, United States), 4 µl undiluted DNA extract (~70 ng - 230 ng DNA), 0.25 µM forward and reverse primers, and nuclease-free H_2O to 20 µl. Thermal cycling conditions for each gene are provided in Table 1. Standard curves for each gene were generated by amplifying serial dilutions of the target genes that were either synthesized (*amoA* genes) or cloned into plasmids (*nirS*, *nirK*, *nosZ*) and standard curves had R^2 values > 0.99. Amplifications were performed in triplicate and amplification specificity was assessed using melt curves. All gene abundances were corrected for dry soil mass and were log transformed prior to analysis.

Data analysis

To test the hypothesized direct and indirect relationships between disturbance intensity, SNF, AM dominance, and soil N variables, we constructed piecewise structural equation models (Lefcheck, 2016). Due to the limitations imposed by our sample size, we constructed structural equation models to assess the relationship between the five core variables: disturbance intensity, hillslope position, SNF, AM dominance, and soil pH and each of our soil N variables. Due to the recent evidence that mycorrhizal type alters the nutrient economy through its effect on pH (G. Lin, Craig, et al., 2022), we tested the hypothesized indirect relationship between disturbance intensity, SNF, and AM dominance on our soil N variables through soil pH. In these models disturbance intensity and hillslope were treated as ordinal variables where values increased with increasing disturbance intensity (selectively cut=1, clearcut=2, and agriculture abandonment=3) and down a hillslope gradient (ridge=1, mid-slope=2, toe-slope=3). We transformed variables

using Tukey's Ladder of Power as necessary. Normality was verified using Shapiro's Test and a visual assessment of residual plots. We tested Zero-inflation and over- or under- dispersion. To investigate the individual patterns between the N transformation rates and N pools, we constructed linear models. For the N cycling gene abundances, we constructed linear mixed effects models where the plate was assigned as a random effect, to account for variability between qPCR runs. When our data was zero-inflated, we fit a binomial logistic regression as piecewise structural equations models are constrained to linear and linear mixed effects models.

CHAPTER 3

RESULTS

We found support for our first hypothesis that increasing intensity of historical land use led to increased abundance of black locust, and hence, cumulative SNF (t=4.44, p <0.001) (Fig. 1, Table 2). Cumulative SNF increased up the hillslope gradient from toe-slope to ridge, suggesting greater abundance and persistence of black locust on ridges following disturbance (t=-2.27, p<0.05) (Fig. 1, Table 2). We also found support for our second hypothesis that higher cumulative SNF promoted the dominance of AM trees over forest recovery (t=3.48, p<0.01) (Fig. 2, Table 2). This suggests that SNF, a natural recovery mechanism, favors AM trees, which are known to promote, and be favored by soils with high N transformation rates. However, disturbance intensity also had a direct positive effect on AM dominance, suggesting that the recruitment of AM tree species may be favored over ECM tree species with increasing disturbance intensity (t=5.97, p<0.001) (Fig. 2, Table 2). In contrast to SNF, AM dominance increased from ridges to toe-slopes (t=1.80, p<0.1) (Fig. 2, Table 2).

Soil pH

Due to the recent evidence that mycorrhizal effects on the N cycle may be mediated through soil acid-base chemistry (G. Lin, Craig, et al., 2022), we included soil pH as a term in our models. We found that increasing AM dominance was associated with higher soil pH (t=2.43, p<0.05) (Fig. 3A, Table 2). Hillslope position also had a direct positive effect on pH, such that it increased from ridges to toe-slopes (t=2.75, p<0.01) (Fig. 3A, Table 2). Thus, our

findings indicate that disturbance and SNF both have positive, indirect effects on pH via their effect on AM dominance (Fig. 3B).

Soil total C and N and inorganic N pools

To test our hypothesis that AM dominance affected the N cycle, we iteratively included soil total C and N and inorganic pools of N in our structural equation models. Overall, we found that most pools of N were indirectly affected by AM dominance through its positive effect on soil pH (Fig. 4B, 5B, 7B). We found that soil pH had a direct positive effect on total C (t=-2.71, p<0.05) (Fig. 4A, Table 2) and a direct negative effect on the C:N ratio (t=-5.19, p<0.001) (Fig. 5A, Table 2). We also found that hillslope position had positive effects on total C (t=2.14, p<0.05) (Fig. 4A, Table 2) and N (t=2.05, p<0.05) (Fig. 6A, Table 2), such that they increased from ridges to toe-slopes, showing the effects of hillslope on the accumulation of organic matter.

Similarly, we found that pH had positive, direct effects on resin dissolved inorganic N (z=2.57, p<0.05) (Fig. 7A, Table 2) and the presence of ammonium (z=1.66, p<0.1) (Fig. 8A, Table 2) and nitrate (z=1.74, p<0.1) (Fig. 9A, Table 2) in resin extractions. Whereas AM dominance had a positive indirect effect on resin N via pH (Fig. 7B, 8B, 9B). In contrast to resin pools, we found that cumulative SNF (t=1.79, p<0.1) (Fig. 10A, Table 2) and pH (t=1.84, p<0.1) (Fig. 10B, Table 2) both had direct, positive effects on the ammonium pool while disturbance intensity had a direct negative effect on the ammonium pool (t=-3.49, p<0.01) (Fig. 10, Table 2). AM dominance also had a positive, indirect effect on ammonium pool via pH (Fig. 10C). The presence of extractable nitrate was not significantly related to any of the variables in our model (Table 2).

N transformation rates

Regarding our hypothesis that AM dominance affected the rate of N cycle transformations, we found that SNF (t=2.77, p<0.01) (Fig. 11A, Table 2) and pH (t=3.48, p<0.01) (Fig. 11B, Table 2) both had positive direct effects on potential mineralization rates, showing the indirect effect of AM dominance (Fig. 11C). Similarly, potential nitrification (presence) was also positively and directly related to SNF (z=2.65, p<0.01) (Fig. 12A, Table 2) and pH (z=2.35, p<0.05) (Fig. 12C, Table 2). However, we found that AM dominance had a direct negative effect on potential nitrification, counter to our hypothesis (z=-1.71, p<0.1) (Fig. 12B, Table 2). Potential nitrification (presence) also increased from ridges to toe-slopes (z=2.08, p<0.05) (Fig. 12, Table 2). Lastly, potential denitrification rates increased with increasing AM tree dominance (t=2.15, p<0.05) (Fig. 13A, Table 2), pH (t=2.26, p<0.05) (Fig. 13B, Table 2), and from ridges to toe-slopes (t=2.50, p<0.05) (Fig. 13, Table 2).

Microbial abundance

To test whether AM tree dominance affected microbial communities responsible for facilitating N cycle transformations, we quantified the abundance of the microbial genes responsible for the rate limiting steps of nitrification (*amoA*) and denitrification (*nirK, nirS, nosZ*). Overall, many genes were indirectly affected by AM tree dominance via the direct effect of pH (Fig. 17B, 19B); however, in two cases, AM tree dominance had direct, negative effects on gene abundances (Fig. 14D, 15B). We found that the abundance of AOA increased with increasing SNF (t=1.6, p<0.1) (Fig. 14A, Table 2) and increasing pH (t=3.86, p<0.01) (Fig. 14C, Table 2), however, counter to our expectation, their abundance decreased with increasing AM dominance (t=-2.17, p<0.05) (Fig. 14B, Table 2). We also found that the abundance of AOB decreased with increasing AM dominance (t=-2.10, p<0.05) (Fig. 15A, Table 2) and increased

with increasing pH (t=4.10, p<0.001) (Fig. 15B, Table 2) and disturbance intensity (t=3.11, p<0.01) (Fig. 15, Table 2). In contrast, the abundance of CAOB was only related to hillslope position, where it increased from ridges to toe-slopes (t=2.41, p<0.05) (Fig. 16A, Table 2).

For genes related to denitrification, the abundance of *nirK* increased with increasing pH (t=6.52, p<0.001) (Fig. 17A, Table 2) and increasing disturbance intensity (t=1.72, p<0.1) (Fig. 17, Table 2), and the abundance of *nirS* increased with increasing disturbance intensity (t=1.81, p<0.1) (Fig. 18A, Table 2) and from ridges to toe-slopes (t=4.32, p<0.001) (Fig. 18, Table 2). The abundance of the *nosZ* gene increases with increasing pH (t=2.99, p<0.01) (Fig. 19A, Table 2) and from ridges to toe-slopes (t=4.53, p<0.001) (Fig. 19, Table 2).

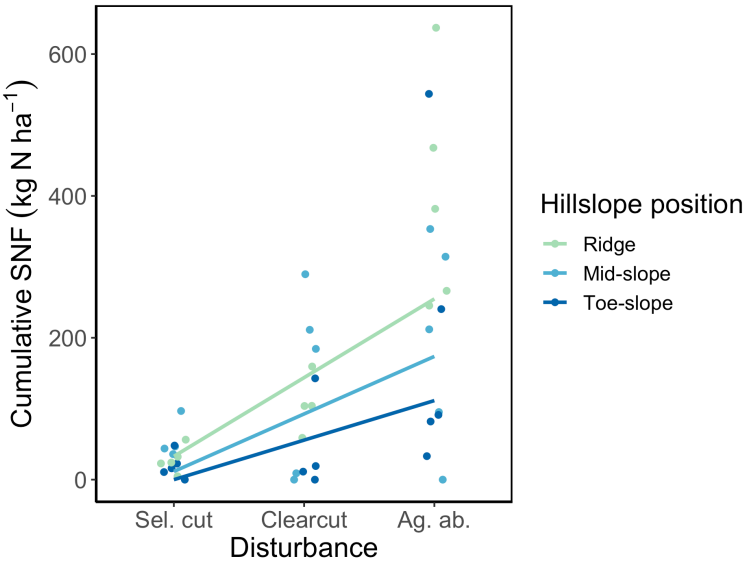


Figure 1. Relationship of cumulative SNF (kg N ha^{-1}) by black locust with increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively) and hillslope position (ridge, mid-slope, and toe-slope). Our predictions are back transformed, and data are untransformed.

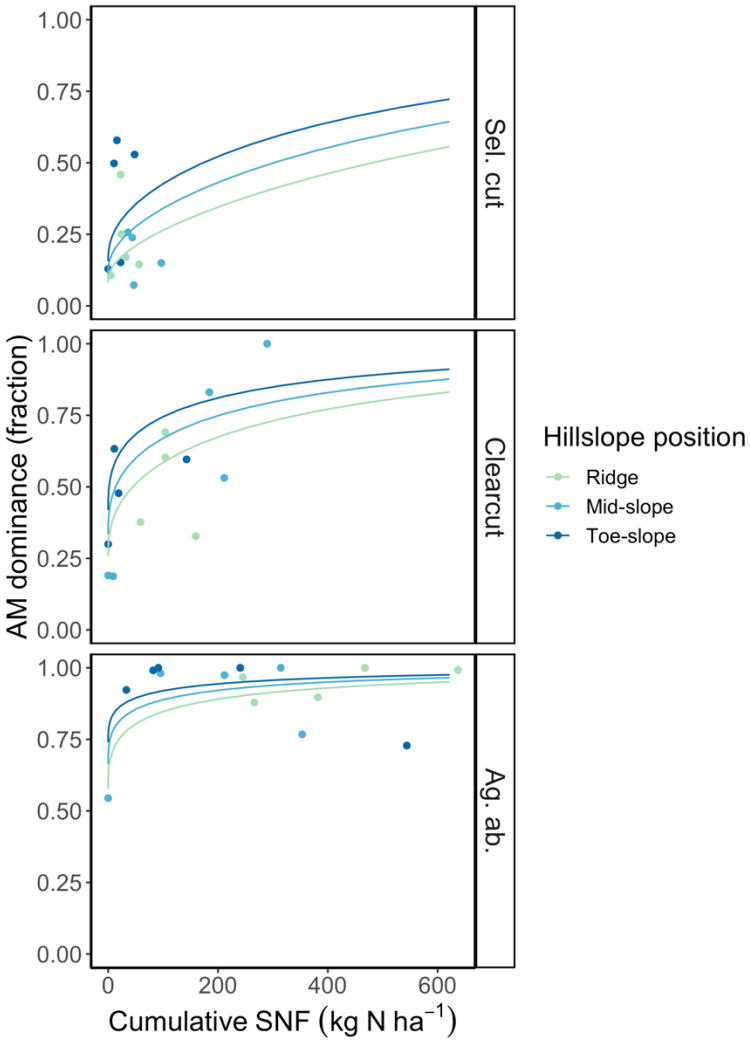


Figure 2. Relationship of AM tree dominance (fraction of total aboveground tree biomass) with cumulative SNF (kg N ha^{-1}) by black locust, increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively), and hillslope position (ridge, mid-slope, and toe-slope). Our predictions are back transformed, and data are untransformed.

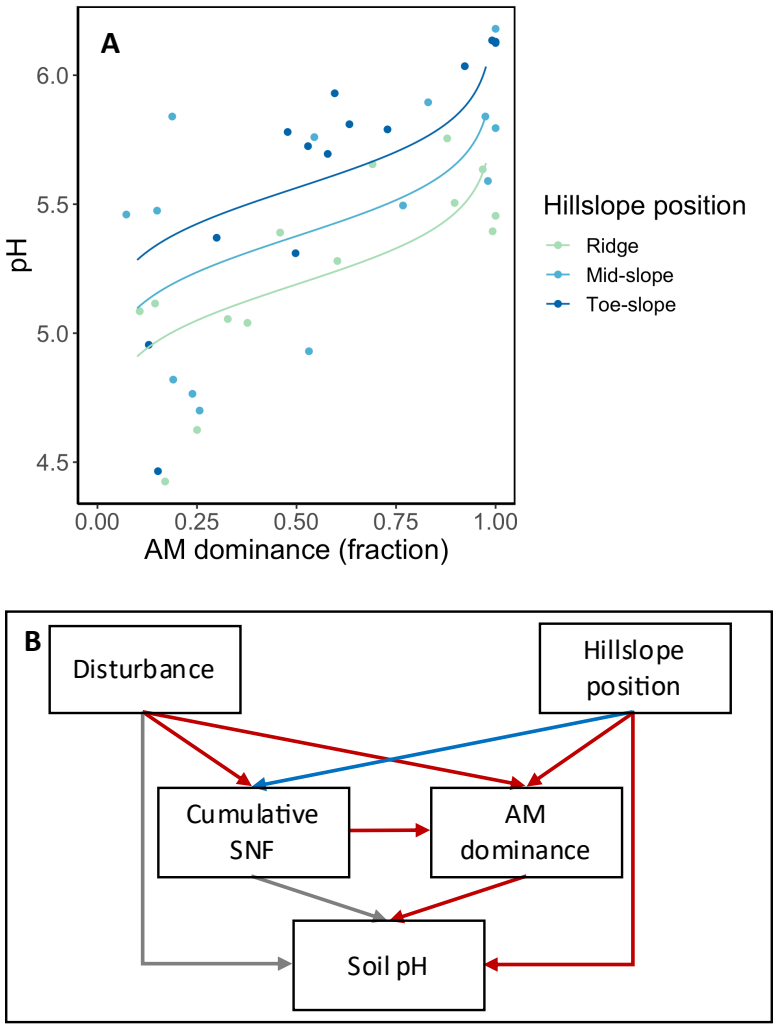


Figure 3. Relationship of soil pH with A) direct influence of AM tree dominance (fraction of total aboveground tree biomass) and hillslope position (ridge, mid-slope, and toe-slope) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, and AM dominance. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

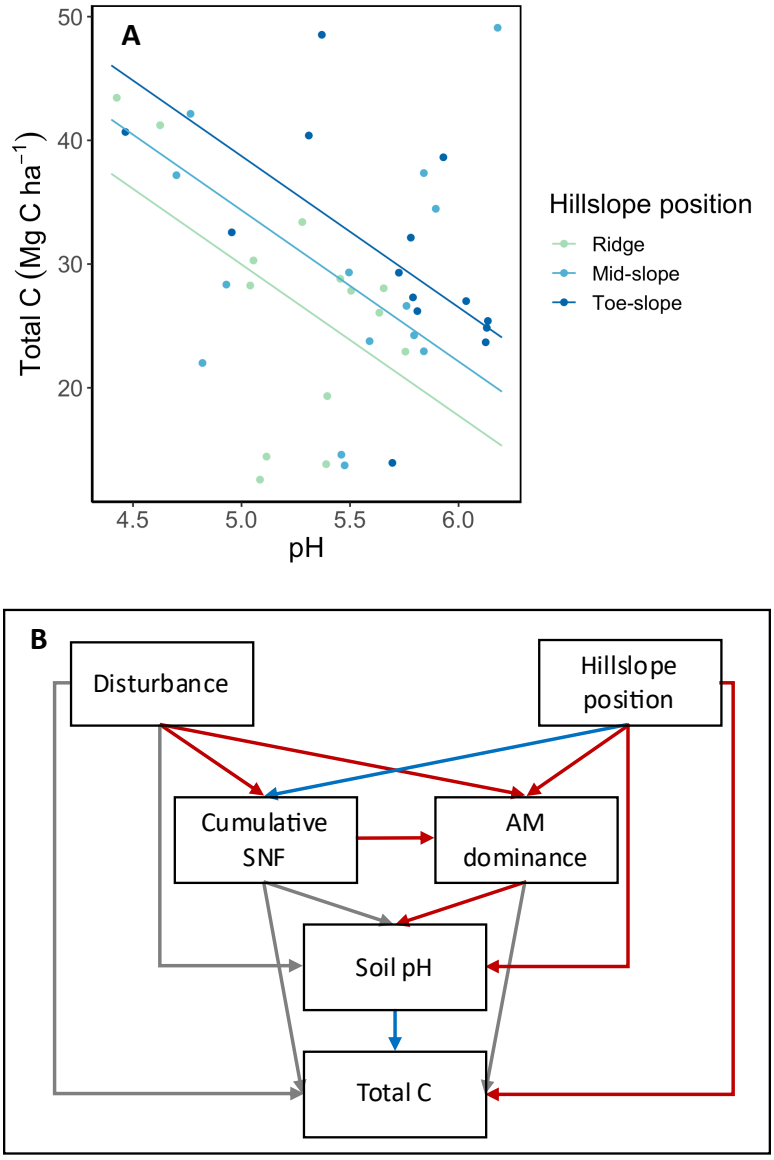
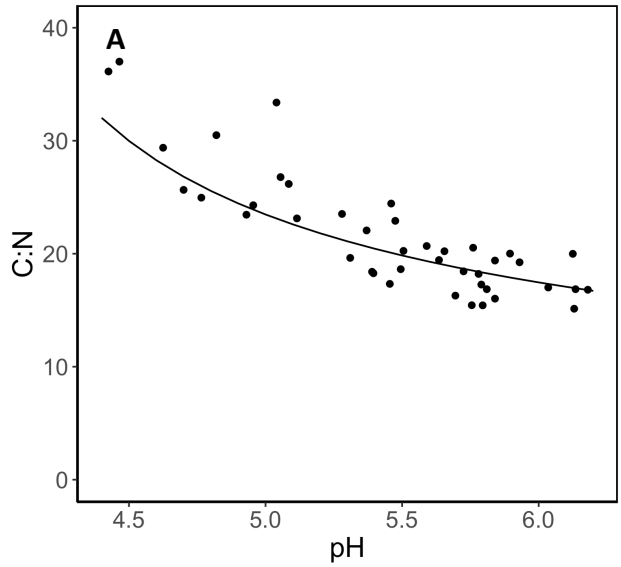


Figure 4. Relationship of total soil C (Mg C ha^{-1}) with A) direct influence of soil pH and hillslope position (ridge, mid-slope, and toe-slope) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.



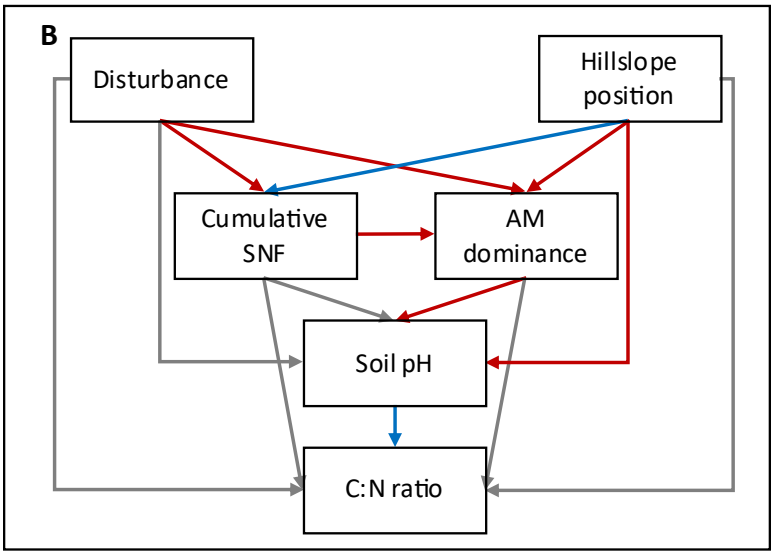


Figure 5. Relationship of soil C:N ratio with A) direct influence of soil pH and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

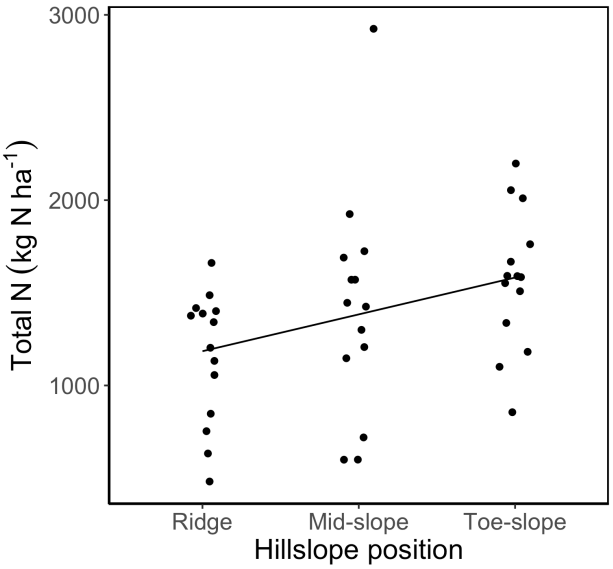


Figure 6. Relationship of total soil N (kg N ha^{-1}) with direct influence of hillslope position (ridge, mid-slope, and toe-slope). Our predictions are back transformed, and data are untransformed.

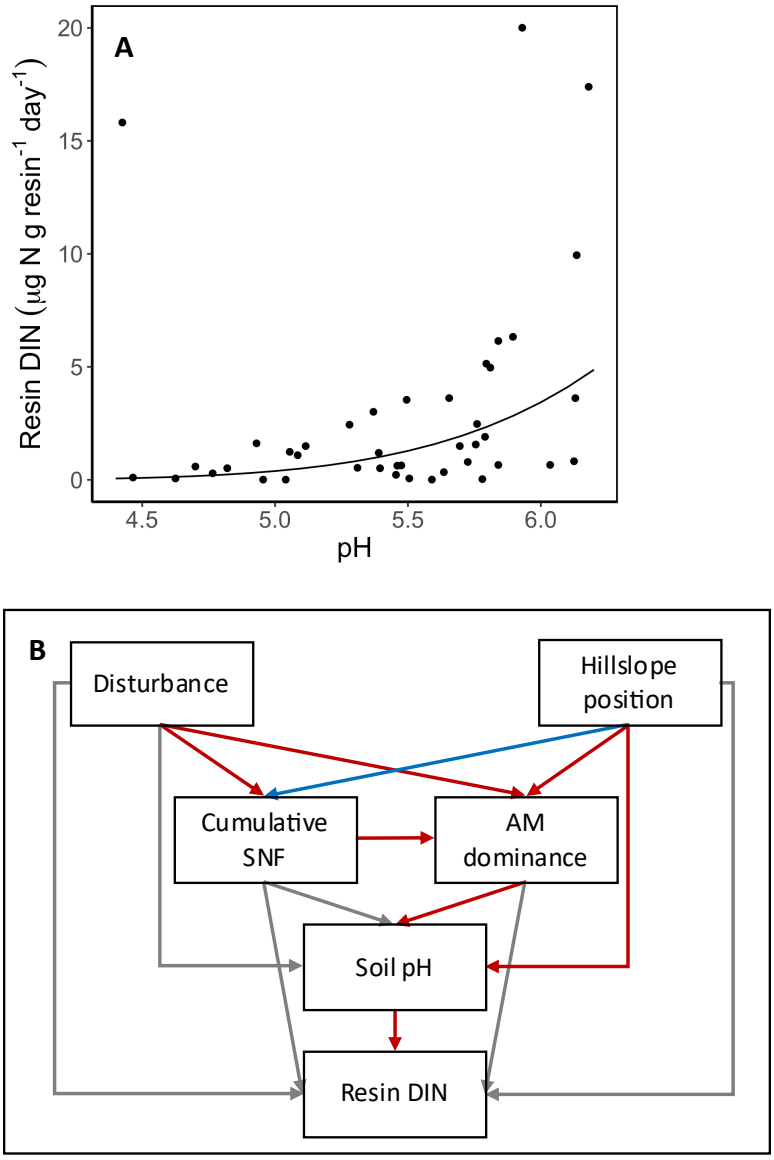


Figure 7. Relationship of resin dissolved inorganic nitrogen (DIN; µg N g resin^{-1} day^{-1}) with A) direct influence of soil pH, B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

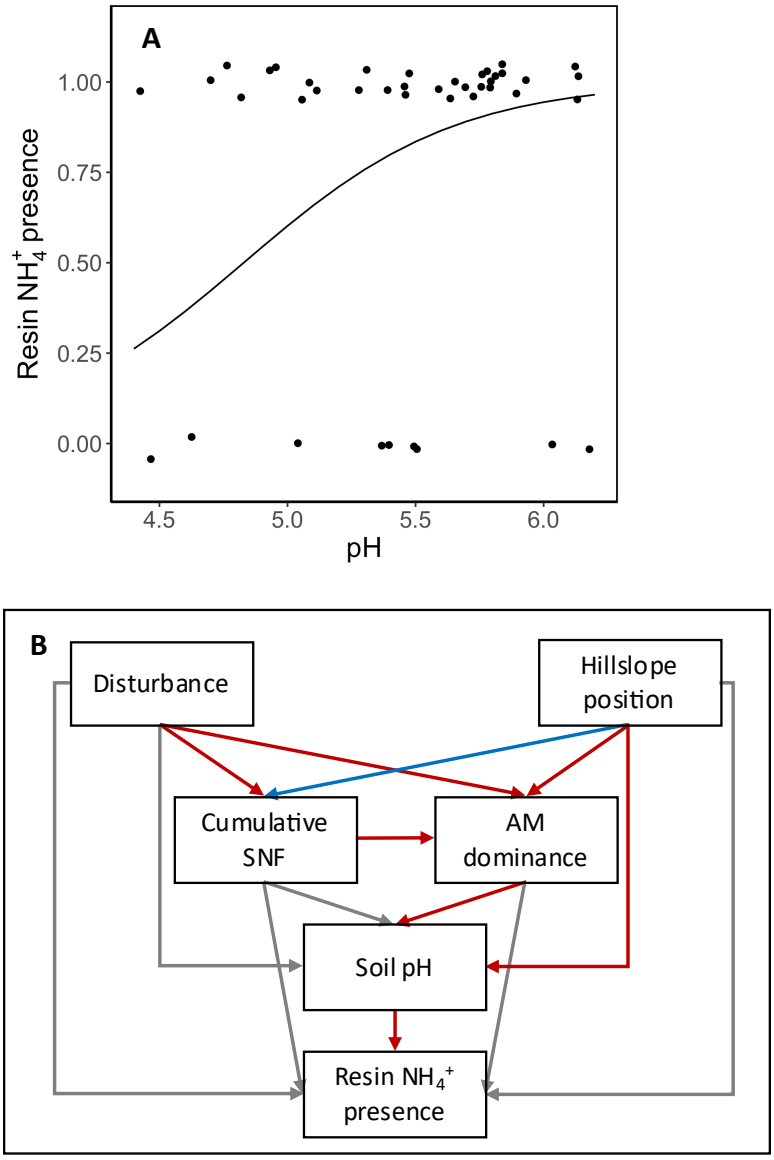


Figure 8. Relationship of resin ammonium (NH_4^+) presence with A) direct influence of soil pH and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

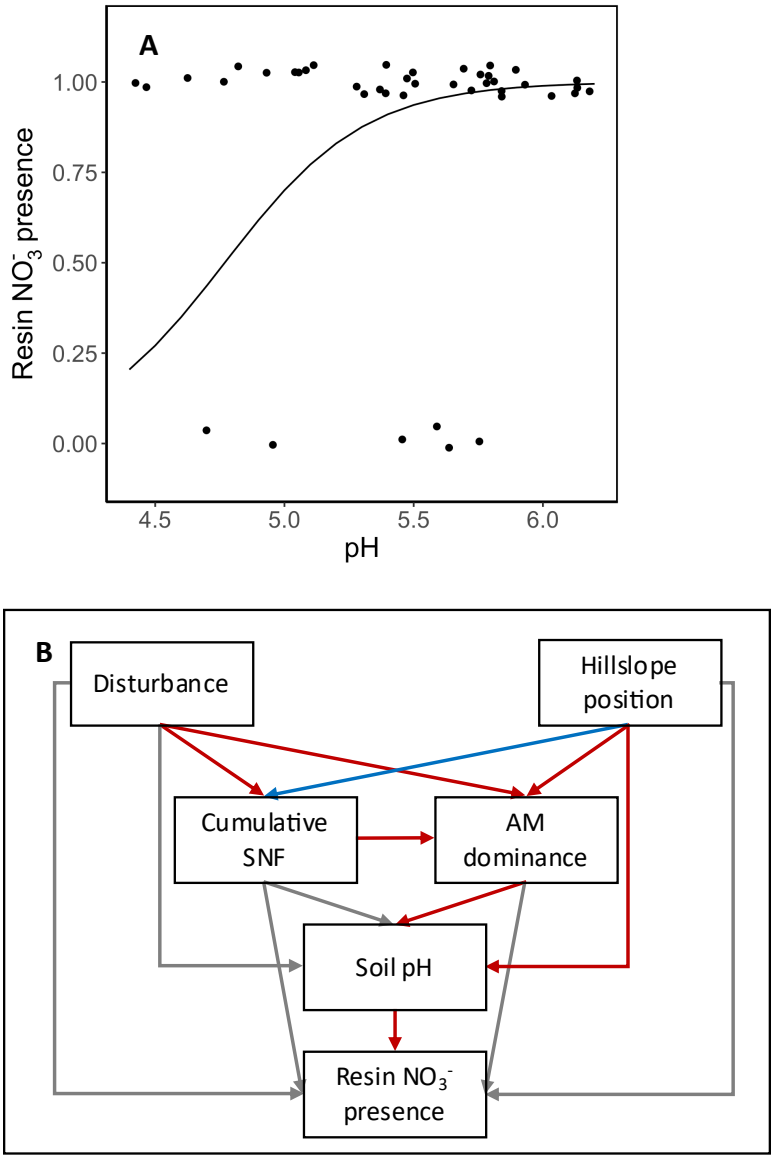


Figure 9. Relationship of resin nitrate (NO_3^-) presence with A) direct influence of soil pH and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

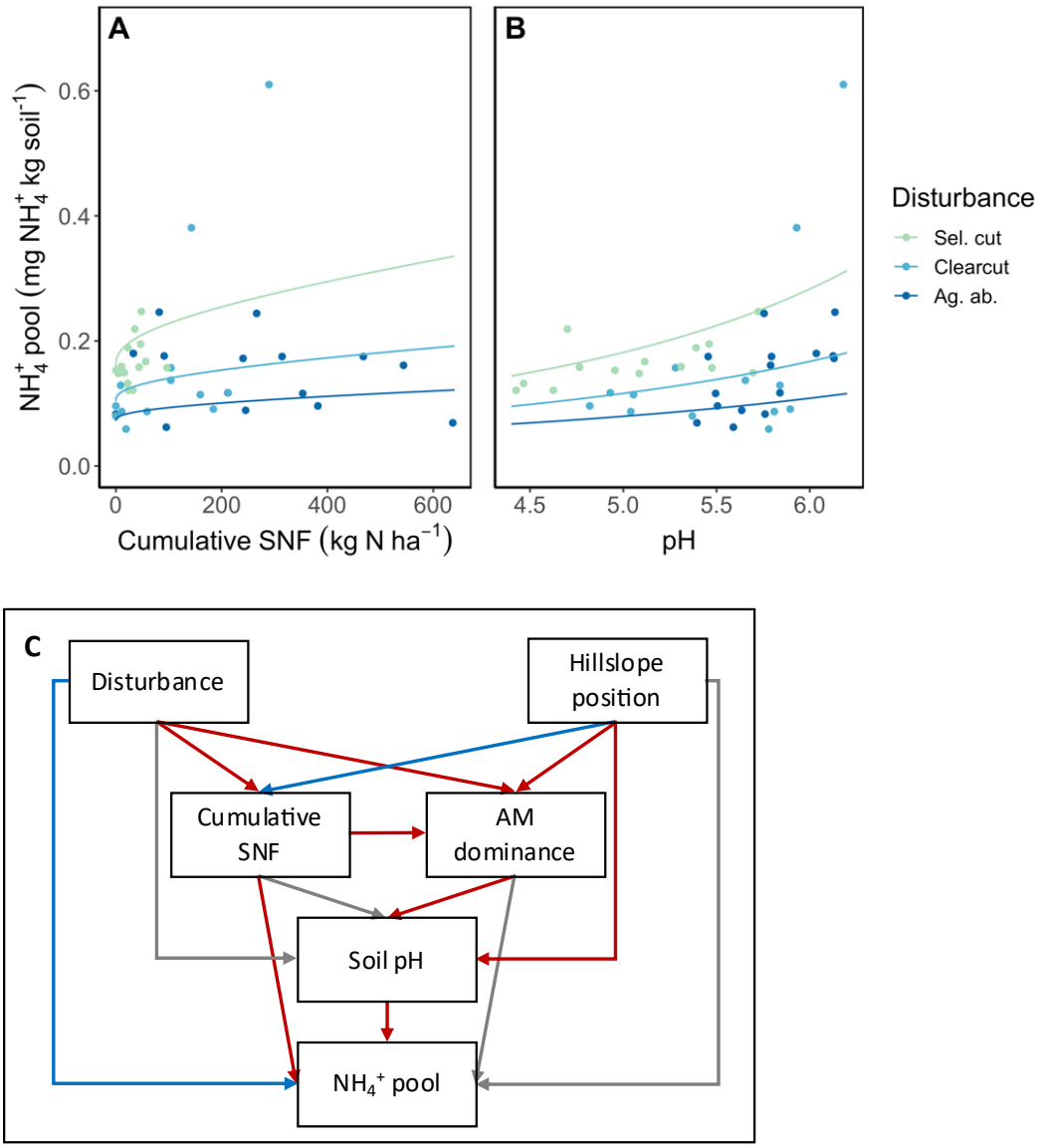


Figure 10. Relationship of ammonium (NH_4^+) pool (mg NH_4^+ kg soil^{-1}) with A) direct influence of cumulative SNF (kg N ha^{-1}) and increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively), B) direct influence of soil pH and increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively), and C) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For C), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray

arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

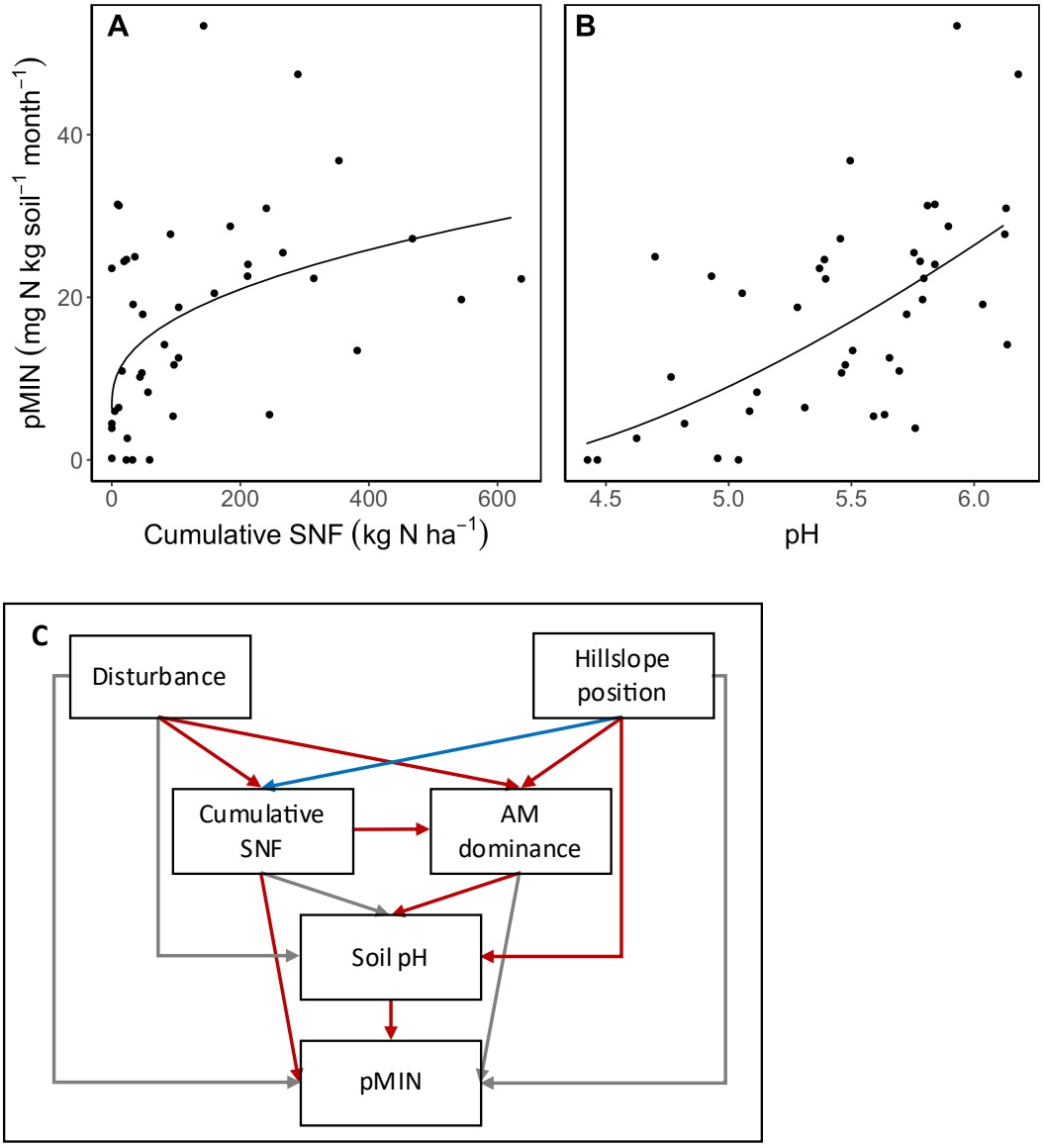


Figure 11. Relationship of potential mineralization (pMIN) rate (mg N kg soil^{-1} month^{-1}) with A) direct influence of cumulative SNF (kg N ha^{-1}), B) direct influence of soil pH, and C) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For C), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

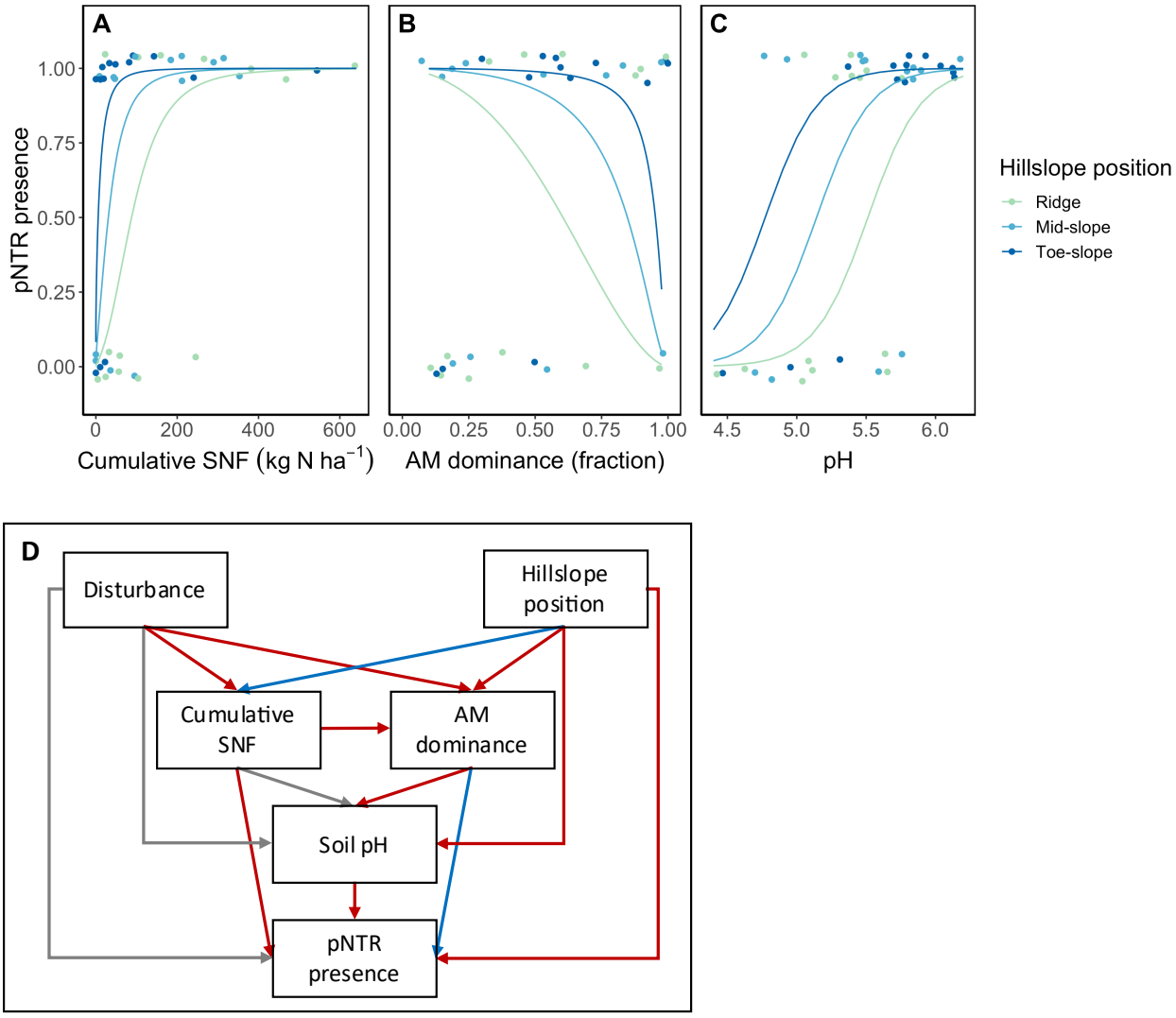


Figure 12. Relationship of potential nitrification (pNTR) presence with A) direct influence of cumulative SNF (kg N ha^{-1}) and hillslope position (ridge, mid-slope, and toe-slope), B) direct influence of AM tree dominance (fraction of total aboveground tree biomass) and hillslope position (ridge, mid-slope, and toe-slope), C) direct influence of soil pH and hillslope position (ridge, mid-slope, and toe-slope), and D) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For D), red arrows indicate a significant, positive directional effect, blue arrows indicate a

significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

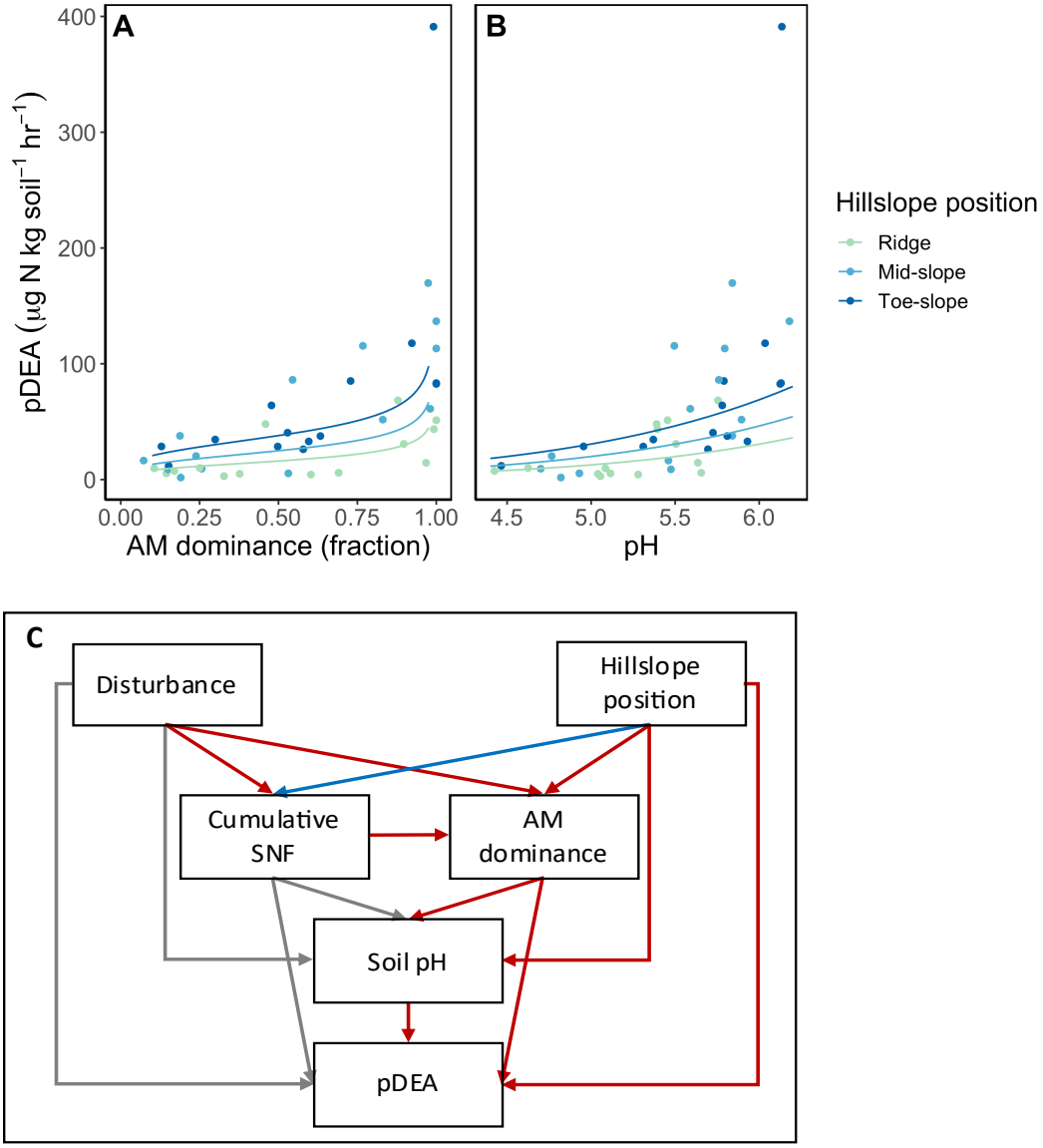


Figure 13. Relationship of potential denitrification (pDEA) rate (µg N kg soil^{-1} hr^{-1}) with A) direct influence of AM tree dominance (fraction of total aboveground tree biomass) and hillslope position (ridge, mid-slope, and toe-slope), B) direct influence of soil pH and hillslope position (ridge, mid-slope, and toe-slope), and C) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For C), red arrows indicate a significant, positive directional effect, blue arrows indicate a

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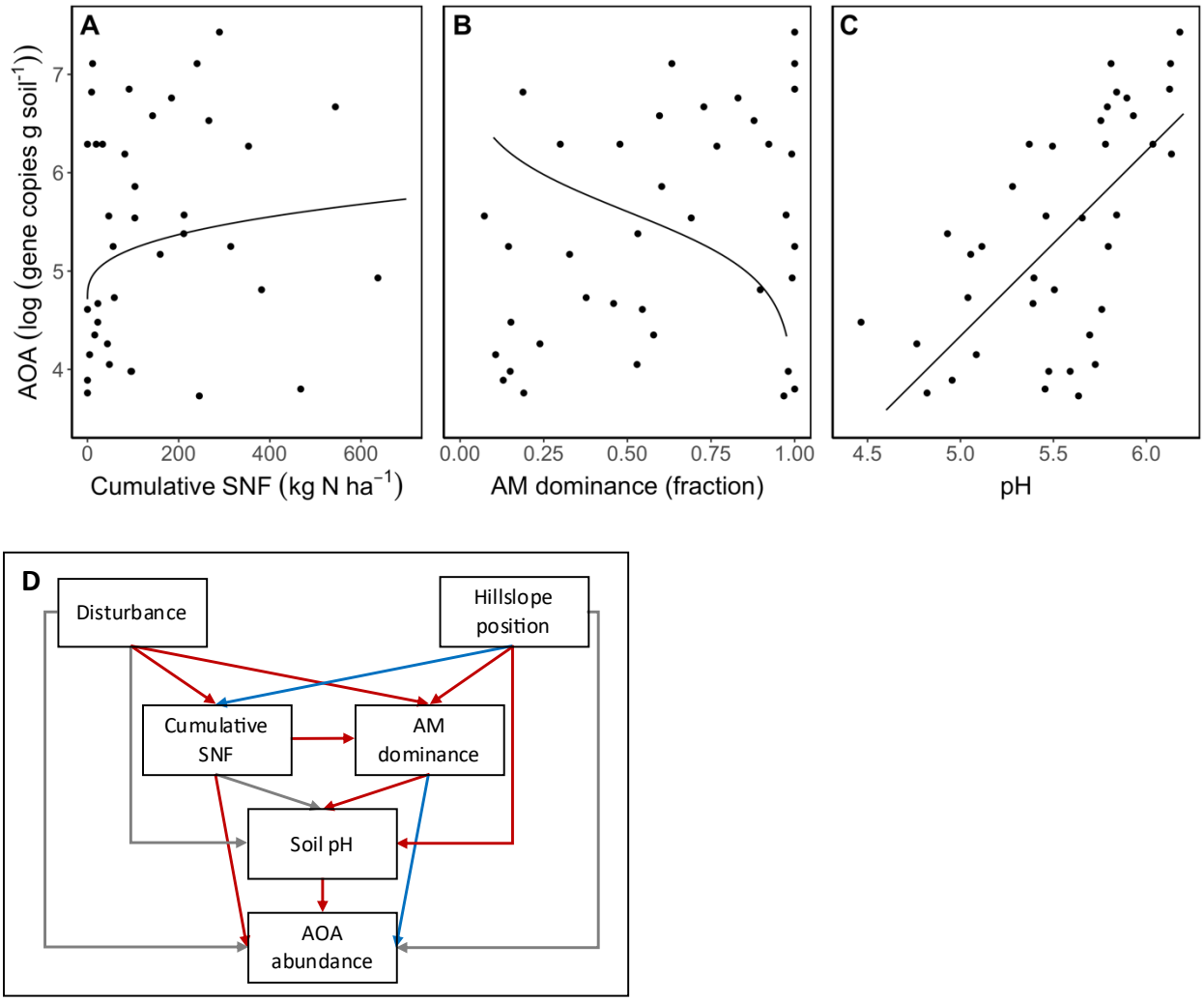

Figure 14. Relationship of ammonia-oxidizing archaea (AOA) abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of cumulative SNF (kg N ha^{-1}), B) direct influence of AM tree dominance (fraction of total aboveground tree biomass), C) direct influence of soil pH, and D) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For D), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

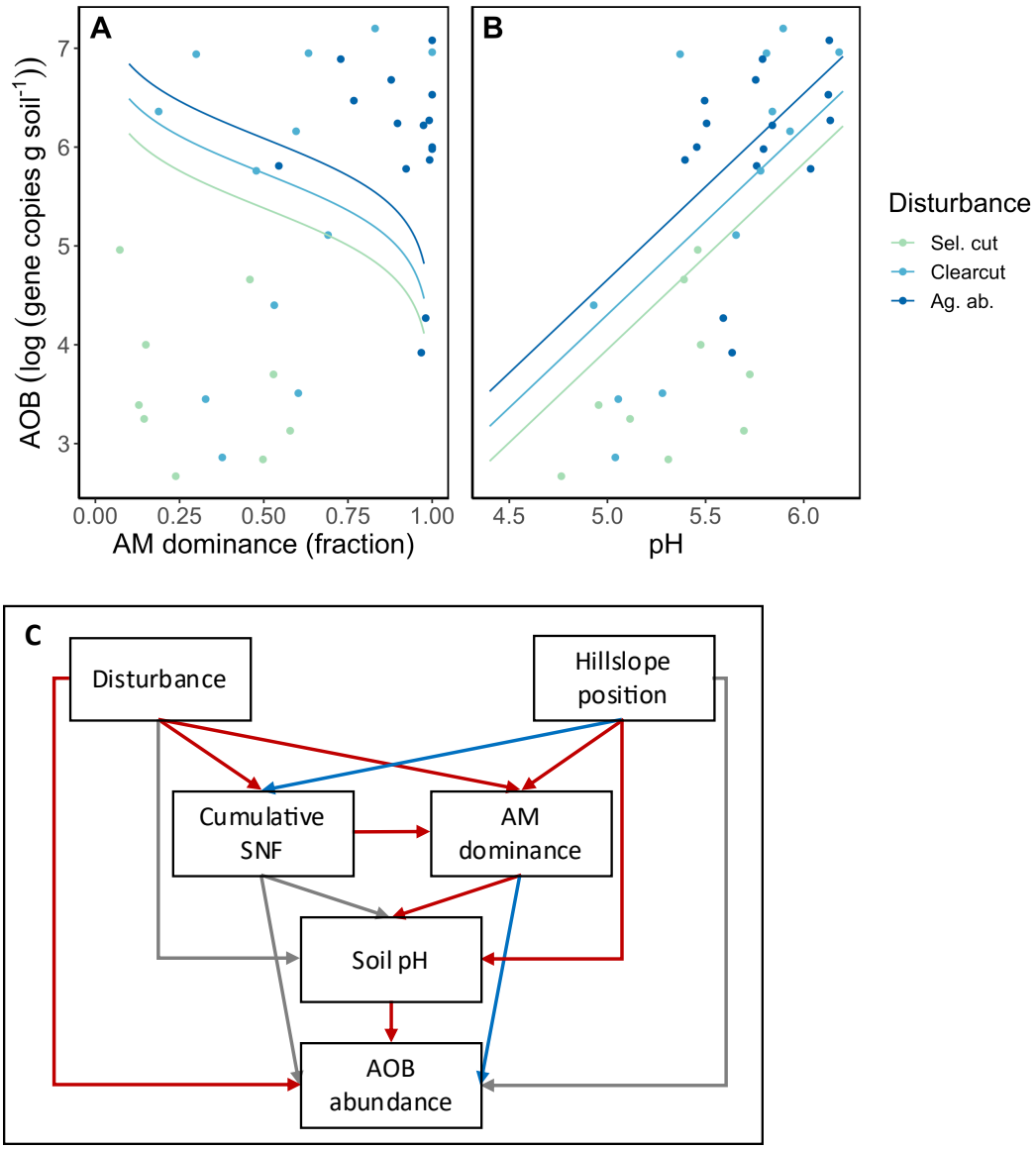

Figure 15. Relationship of ammonia-oxidizing bacteria (AOB) abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of AM tree dominance (fraction of total aboveground tree biomass) and increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively), B) direct influence of soil pH and increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively), and C) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For C), red arrows indicate a significant, positive directional

effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

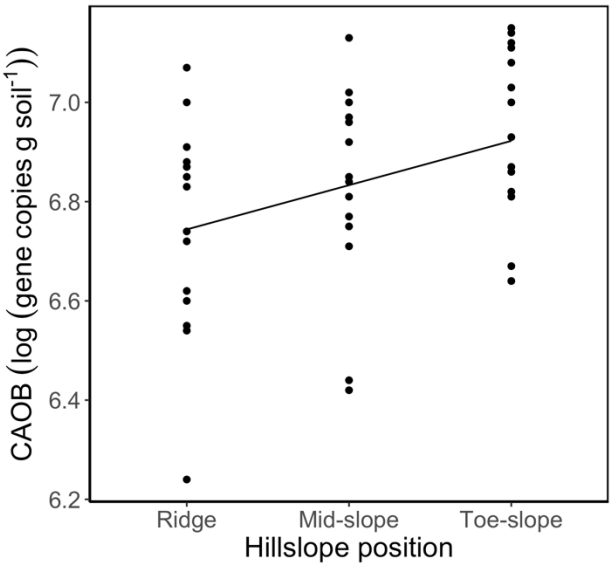

Figure 16. Relationship of complete ammonia-oxidizing bacteria (CAOB) abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of hillslope position (ridge, mid-slope, and toe-slope) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH.

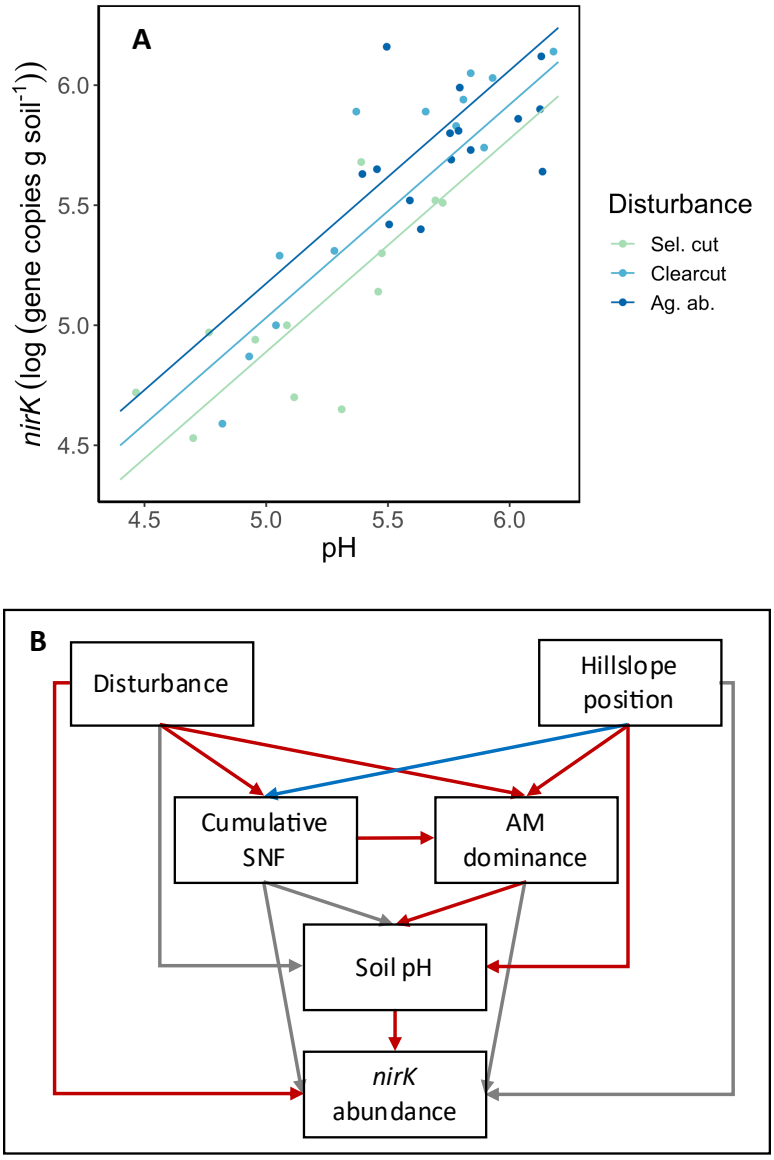

Figure 17. Relationship of *nirK* gene abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of soil pH and increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

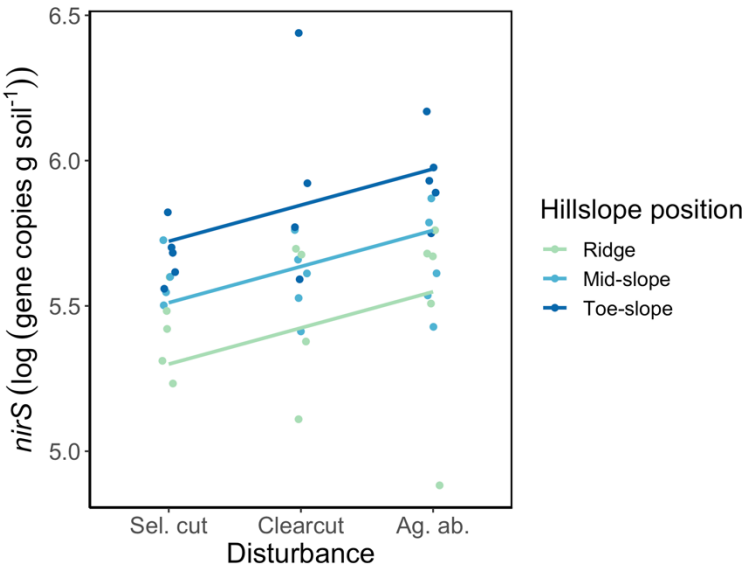

Figure 18. Relationship of *nirS* gene abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of increasing disturbance intensity (selectively cut, clearcut, and agriculture abandonment, respectively) and hillslope position (ridge, mid-slope, and toe-slope) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

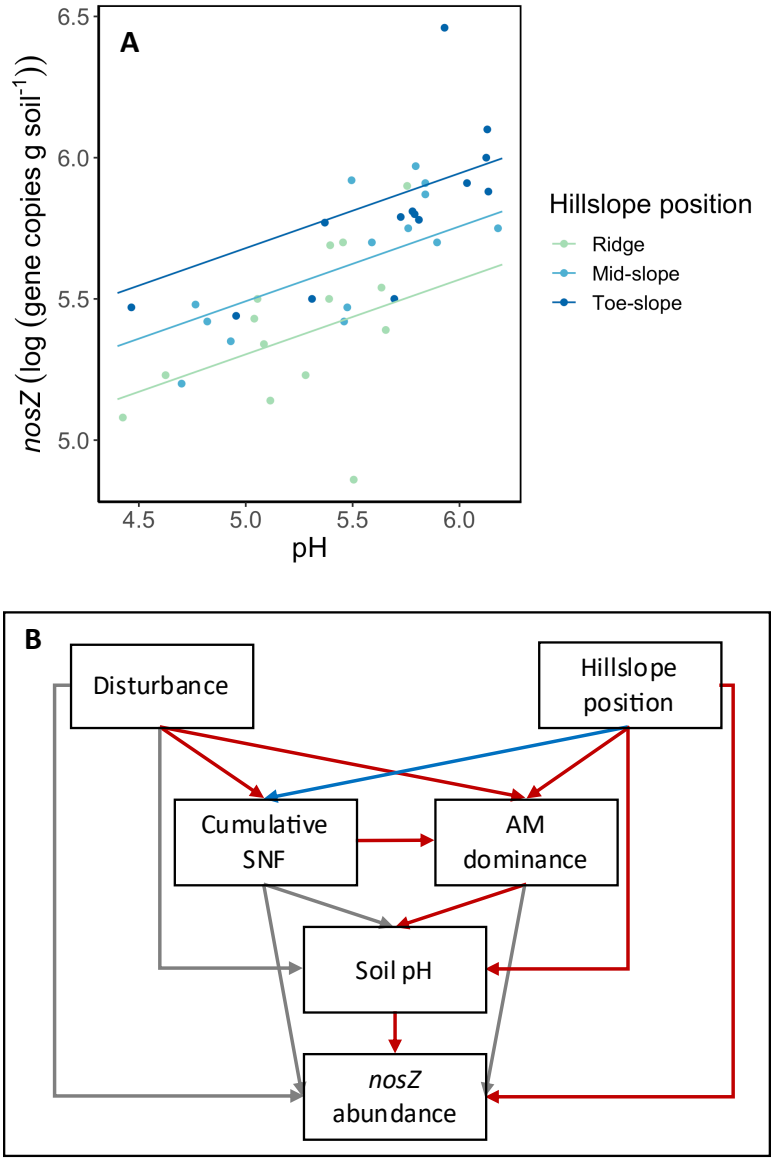

Figure 19. Relationship of *nosZ* gene abundance (log(gene copies g^{-1} $soil^{-1}$) with A) direct influence of soil pH and hillslope position (ridge, mid-slope, and toe-slope) and B) indirect and direct influences of each variable in our models: disturbance intensity, hillslope position, SNF by black locust, AM dominance, and soil pH. For B), red arrows indicate a significant, positive directional effect, blue arrows indicate a significant, negative directional effect, and gray arrows are insignificant relationships that were tested. Our predictions are back transformed, and data are untransformed.

Table 1: Thermal cycling conditions for nitrification and denitrification genes quantified for this study.

Gene target	Primer Pair	Thermal cycling protocol	qPCR Efficiency
nirS	nirScd3af/nirSR3cd (Throbäck et al., 2004)	15 min at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C.	80.8 – 82.7%
nirK	nirK876/nirK1040 (Henry et al., 2004)	15 min at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 58 °C, 30 s at 72 °C, and 5 s at 80 °C	69.4 – 72.5%
nosZ	nosZ2F/R (Henry et al., 2006)	15 min at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C.	80.2 – 82.2%
AOA *amoA*	Arch-amoAF/R (Francis et al., 2005)	15 min at 95 °C, 40 cycles of 15 s at 94 °C, 30 s at 53 °C, and 60 s at 72 °C.	82.6 – 83.5 %
AOB *amoA*	amoA-1f/2r (Rotthauwe et al., 1997b)	15 min at 95 °C, 40 cycles of 15 s at 94 °C, 30 s at 55 °C, 60 s at 72 °C, and 5s at 78 °C (plate read step).	72.5 – 80.3%
CAOB *amoA*	comamoA F/R (Zhao et al., 2019)	15 min at 95 °C, 40 cycles of 30 s at 94 °C, 30 s at 53 °C, and 60 s at 72 °C.	82.1 – 87.8%

Table 2. Results from linear and logistic models of our N cycle variables and disturbance intensity (Disturbance) and hillslope position (Position) and, where applicable, cumulative symbiotic nitrogen fixation (SNF), arbuscular mycorrhizal dominance (AM), and soil pH. Transformations are shown on the model structure where Tukey transformations are displayed as superscripts where applicable. Only significant relationships ($p<0.1$) are shown.

Variable	Estimate	Test stat	Pr (>|t|)	Marginal R^2	Conditional R^2
Symbiotic nitrogen fixation (kg N ha^{-1})				lm($y^{0.35}$, Family=Gaussian)	
Disturbance	1.658	t=4.438	<0.001	0.38	
Position	-0.863	t=-2.271	<0.05	0.38	
Arbuscular mycorrhizal dominance (fraction)				lm(logit(y), Family=Gaussian)	
Disturbance	1.368	t=5.970	<0.001	0.73	
Position	0.364	t=1.801	<0.1	0.73	
SNF	0.279	t=3.484	<0.01	0.73	
pH				lm(y, Family =Gaussian)	
Disturbance	0.116	t=1.132		0.55	
Position	0.187	t=2.753	<0.01	0.55	
SNF	-0.007	t=-0.228		0.55	
AM	0.127	t=2.430	<0.05	0.55	
Total carbon (Mg C ha^{-1})				lm(y, Family=Gaussian)	
Disturbance	-0.941	t=-0.328		0.19	
Position	4.379	t=2.139	<0.05	0.19	
SNF	-0.033	t=-0.041		0.19	
AM	2.343	t=1.515		0.19	
pH	-12.222	t=-2.708	<0.05	0.19	
Total nitrogen (kg N ha^{-1})				lm(y, Family=Gaussian)	
Disturbance	-87.410	t=-0.643		0.29	
Position	198.740	t=2.052	<0.05	0.29	
SNF	13.230	t=0.343		0.29	
AM	122.380	t=1.673		0.29	
pH	25.210	t=0.118		0.29	
Carbon:nitrogen ratio				lm($-y^{-1.85}$, Family=Gaussian)	
Disturbance	1.78E-04	t=0.685		0.69	
Position	-1.03E-04	t=-0.554		0.69	
SNF	-5.88E-05	t=-0.796		0.69	
AM	-1.51E-04	t=-1.078		0.69	
pH	-2.12E-03	t=-5.192	<0.001	0.69	

Dissolved inorganic nitrogen availability (μg N g resin^{-1} day^{-1})				lm(y$^{0.175}$, Family=Gaussian)
	Disturbance	-0.072	t=-0.742	0.18
	Position	-0.018	t=-0.260	0.18
	SNF	0.021	t=0.776	0.18
	AM	-0.032	t=-0.612	0.18
	pH	0.393	t=2.571 <0.05	0.18
Ammonium availability (μg NH_4^+ g resin^{-1} day^{-1})				glm(y$^{0.375}$, Family=Binomial)
	Disturbance	-0.841	z=-0.885	0.19
	Position	0.293	z=-0.492	0.19
	SNF	-0.033	z=-0.144	0.19
	AM	-0.214	z=-0.455	0.19
	pH	2.415	z = 1.656 <0.1	0.19
Nitrate availability (μg NO_3^- g resin^{-1} day^{-1})				glm(y$^{0.25}$, Family=Binomial)
	Disturbance	-0.299	z=-0.240	0.46
	Position	0.577	z=0.667	0.46
	SNF	0.478	z=1.183	0.46
	AM	-1.310	z=-1.539	0.46
	pH	3.680	z=1.737 <0.1	0.46
Ammonium pool (mg NH_4^+ kg soil^{-1})				lm(-y$^{-0.4}$, Family=Gaussian)
	Disturbance	-0.388	t=-3.486 <0.01	0.30
	Position	0.042	t=0.528	0.30
	SNF	0.056	t=1.786 <0.1	0.30
	AM	0.047	t=0.792	0.30
	pH	0.321	t=1.838 <0.1	0.30
Nitrate pool probability (mg NO_3^- kg soil^{-1})				glm(y, Family=Binomial)
	Disturbance	1.314	z=1.607	0.31
	Position	-0.623	z=-1.020	0.31
	SNF	-0.001	z=-0.006	0.31
	AM	-0.422	z=-1.053	0.31
	pH	1.965	z=1.277	0.31
Potential mineralization (mg N kg soil^{-1} month^{-1})				lm(y$^{0.7}$, Family=Gaussian)
	Disturbance	-0.550	t=-0.575	0.47
	Position	0.576	t=0.844	0.47
	SNF	0.753	t=2.772 <0.01	0.47
	AM	-0.416	t=-0.807	0.47
	pH	5.229	t=3.477 <0.01	0.47
Potential nitrification probability				glm(y, Family=Binomial)
	Disturbance	0.276	z=0.216	0.77
	Position	1.943	z=2.083 <0.05	0.77
	SNF	1.315	z=2.647 <0.01	0.77

AM	-1.507	z=-1.706	<0.1	0.77	
pH	5.252	z=2.347	<0.05	0.77	
Potential denitrification (μg N kg soil^{-1} hour^{-1})				lm(y$^{0.1}$, Family=Gaussian)	
Disturbance	0.012	t=0.363		0.65	
Position	0.060	t=2.500	<0.05	0.65	
SNF	-0.004	t=-0.447		0.65	
AM	0.039	t=2.148	<0.05	0.65	
pH	0.119	t=2.260	<0.05	0.65	
ammonia-oxidizing archaea abundance (log(gene copies g soil^{-1}))				lm(y, Family=Gaussian)	
Disturbance	0.353	t=1.197		0.46	
Position	0.319	t=1.559		0.46	
SNF	0.103	t=1.763	<0.1	0.46	
AM	-0.343	t=-2.170	<0.05	0.46	
pH	1.883	t=3.860	<0.01	0.46	
ammonia-oxidizing bacteria abundance (log(gene copies g soil^{-1}))				lm(y, Family=Gaussian)	
Disturbance	0.967	t=3.112	<0.01	0.64	
Position	0.173	t=0.723		0.64	
SNF	0.100	t=1.535		0.64	
AM	-0.343	t=-2.103	<0.05	0.64	
pH	2.550	t=4.103	<0.001	0.64	
comammox bacteria abundance (log(gene copies g soil^{-1}))				lme(y, Family=Gaussian)	
Disturbance	0.029	t=0.582		0.15	0.58
Position	0.089	t=2.410	<0.05	0.15	0.58
SNF	0.008	t=0.591		0.15	0.58
AM	-0.045	t=-1.657		0.15	0.58
pH	-0.071	t=-0.899		0.15	0.58
nirK abundance (log(gene copies g soil^{-1}))				lme(y, Family=Gaussian)	
Disturbance	0.142	t=1.718	<0.1	0.72	0.73
Position	0.007	t=0.124		0.72	0.73
SNF	0.020	t=0.982		0.72	0.73
AM	-0.054	t=-1.192		0.72	0.73
pH	0.888	t=6.523	<0.001	0.72	0.73
nirS abundance (log(gene copies g soil^{-1}))				lm(y, Family=Gaussian)	
Disturbance	0.125	t=1.813	<0.1	0.41	
Position	0.211	t=4.316	<0.001	0.41	
SNF	0.017	t=0.878		0.41	
AM	-0.048	t=-1.289		0.41	
pH	0.012	t=0.113		0.41	
nosZ abundance (log(gene copies g soil^{-1}))				lme(y, Family=Gaussian)	
Disturbance	0.083	t=1.458		0.60	0.74

	Position	0.188	t=4.531	<0.001	0.60	0.74
	SNF	0.010	t=0.634		0.60	0.74
	AM	-0.003	t=-0.089		0.60	0.74
	pH	0.264967	t=2.994	<0.01	0.60	0.74

CHAPTER 4

DISCUSSION

We investigated a mechanism by which land use disturbance could have long-term effects on the N cycle –the N fixer founder effect. We found that, generally, disturbance-induced SNF promoted AM tree dominance, and this led to increased N transformation rates, soil N pools, and greater abundances of N cycling genes. The effects of AM dominance on our N cycle variables was generally indirect and manifested through higher soil pH.

SNF

We found support for our hypothesis that increasing disturbance intensity increases cumulative SNF. While prior studies have shown that SNF increases following disturbance and then declines in later successional stages (Boring et al., 1988), we found that cumulative SNF increased with increasing intensity of disturbance, an observation that depends on long-term data. Greater cumulative SNF with increasing disturbance intensity is likely explained by the same mechanisms that restrict N fixers to early-succession in temperate forests: N-limitation and high-light availability favor species capable of N fixation relative to those that cannot (Menge et al., 2010). We also found that cumulative SNF increased up a hillslope gradient, which may coincide with increased light availability due to greater canopy exposure, or lower soil nutrient availability due to shallower, drier soils. As above, these local conditions may favor N fixers over non-fixers and explain our estimates of high cumulative SNF on ridges relative to mid- and toe-slope positions.

AM dominance

We also found support for our hypothesis that increasing historical, disturbance-induced SNF increases AM dominance. This suggests that historical SNF promoted high N availability locally, which favored the growth of AM trees over ECM trees. Our finding is consistent with the theoretical analysis of Lu & Hedin (2019), which suggests that AM and ECM associations represent alternative stable states in temperate forests. Transitions in mycorrhizal dominance are possible if the nutrient regime changes, as could occur with local enrichment of soil N through SNF. Prior work has focused on how fire-exclusion and N deposition have promoted the dominance of AM trees in eastern forests of the United States (Averill et al. 2018, Jo et al., 2019), and our work suggests that disturbance-induced SNF also plays a role. Our findings also suggest that increasing disturbance intensity directly promotes AM abundance. AM associated species such as *A. rubrum* and *L. tulipifera* tend to be opportunistic and dominate early successional stages (Elliott et al., 1997). But these species also have the potential to become forest dominants even decades after disturbance (Beck & Hooper, 1986), consistent with what we found in our most disturbed watershed. We also found that AM dominance is highest in the toe-slopes. AM trees may be more favored in toe-slopes because these soils have higher nutrient and water availability (Elliott et al., 2020; Teste et al., 2020), due to the mobility of nutrients downslope (Knoepp & Clinton, 2009), and high moisture availability favoring microbial activity (Orchard & Cook, 1983). In addition, many AM trees have high transpiration rates and tend to be found in coves or riparian areas (Elliott et al., 2017, 2020; Ford et al., 2011). Thus, the local dominance of AM tree species may be explained by historical disturbances, as well as local moisture and nutrient conditions that most favor their competitive success.

pH

In our system, where AM dominance has been increasing for decades (Elliott et al., 1997; Elliott & Swank, 1994b), we have evidence that higher soil pH coincides with higher AM dominance, which may be the result of the absence of ECM trees and their acidifying properties. Both ECM fungi and ECM trees have properties that could lead to soil acidification. ECM fungi produce organic acid root exudates (Yin et al., 2014) and ECM trees produce slow-decomposing leaf litter that diminishes base cation return to the soil thus decreasing acid-buffering capacities (Deano & Robinson, 1985; Hobbie et al., 2006; Keller & Phillips, 2019). We also found that topographic position directly influences soil pH, where soil pH increases down a hillslope gradient. This pH gradient could be the result of differences in soil genesis and age (Chadwick & Chorover, 2001; Husson, 2013), with lower soil pH on ridge tops where soils tend to be more shallow and poorly developed (Losche et al., 1970). Similarly, a study by Sariyildiz et al., 2005 found that pH increased down a hillslope gradient and that pattern coincided with leaf litter quality. In our system, AM associated species are associated with higher leaf litter quality (Phillips et al., 2013), these tree species are more abundant in toe-slope soils, which coincides with the pattern in pH we observed.

Soil total C and N and inorganic N pools

We did not find a direct effect of AM dominance on total soil C or C:N ratio which is in contrast to studies that have shown that AM dominance is associated with lower total C or C:N (Averill et al., 2014; G. Lin, Yuan, et al., 2022; Zhu et al., 2018). However, we did find that pH had a significant, negative effect on both, suggesting that mycorrhizal dominance indirectly affects total C and C:N through its effect on soil pH. Total C in this system is primarily composed of organic C, therefore the relationship of increasing pH leading to lower total C and

C:N could be a result of leaf litter quality. AM trees have higher leaf litter quality (Midgley et al., 2015), which promotes microbial decomposition, the loss of soil C and the reduction of soil C:N. In contrast, total N increased down the hillslope gradient likely due to soil nutrient mobility (as above).

Contrary to our expectations, we did not find that disturbance-induced SNF or mycorrhizal dominance had direct effects on pools of extractable ammonium and nitrate in the landscape (Boring & Swank, 1984; Phillips et al., 2013). However, we did see the indirect effect of AM dominance on resin extractable DIN, nitrate, and ammonium via the relationship between AM dominance and soil pH, consistent with the idea that AM-dominated stands tend to promote an inorganic nutrient economy (Phillips et al., 2013). Further, we found that the KCl-extractable ammonium pool was negatively associated with increasing disturbance, but was positively associated with SNF and pH.

N transformation rates

Overall, we found that soil pH was positively associated with potential mineralization, nitrification (presence) and denitrification rates, which indirectly supports our hypothesis that AM dominance promotes increased N transformation rates. Recent work suggests that AM trees promote rapid N transformation rates through their effect on soil pH (Lin et al., 2022), and our findings are consistent with this framework. We found that potential mineralization and potential nitrification increased with increasing cumulative SNF, likely due to increased N inputs via SNF. However, we observed direct effects of AM dominance that differed in direction – we observed a negative relationship between AM dominance and the presence of potential nitrification and a positive relationship between AM dominance and potential denitrification rates. Due to the immobility of the substrates, ammonium and organic N, nitrification and mineralization may

occur where the N source originates whereas nitrate, the substrate needed for denitrification, may be more susceptible to abiotic controls due to its high mobility in soils (Vitousek et al., 1982). One N source for nitrification may be foliar N, which has been shown to increase net nitrification (Garten, 1993), and is higher in black locust than our dominant AM trees (*A. rubrum* and *L. tulipifera*). Conversely, increased soil moisture favors both potential denitrification and AM dominance in the landscape and could lead to the spatial coupling (Elliott & Swank, 1994a; Maag & Vinther, 1996).

N cycling gene abundance

We found mixed support for our hypotheses regarding nitrifier abundance. Both AOA and AOB abundance decreased with increasing AM dominance. Similarly, a mesocosm experiment by Chen et al., 2013 found that AM fungi negatively impacted AOA and AOB abundance within the rhizosphere. While our study took place at the landscape scale rather than in the rhizosphere, similar mechanisms could be involved. One possible explanation for our finding is that AM fungi may derive more of their N demand from ammonium than nitrate (Tanaka & Yano, 2005), suggesting potential competition for N between AM fungi and AOA and AOB. However, we did not observe a similar pattern with CAOB, which would be consistent with that line of reasoning. We also found that AOA and AOB abundance increased with increasing pH, providing indirect evidence that AM dominance promotes nitrifiers. Other studies have found that the effect of pH on nitrifiers depends on whether they are archaea or bacteria (Nicol et al., 2008), however, we found similar relationships between soil pH on both AOA and AOB. Nitrification and nitrifiers are generally more prevalent in more-basic soils than in acidic soils—nitrification and nitrifier abundance is diminished in highly acidic soils overall (De Boer & Kowalchuk, 2001; Osburn & Barrett, 2020; Prosser & Nicol, 2012; G. P. Robertson, 1982).

We also found that AOA abundance increased with increasing cumulative SNF directly, consistent with our finding that SNF promotes mineralization, creating the substrate for AOA. AOA, unlike AOB, are also able to assimilate organic substrates other than ammonia (Tourna et al., 2011; Walker et al., 2010). Generally, substrate availability is considered a key niche differentiator between AOA and AOB where AOA is more prevalent in low ammonium and acidic soils (Martens-Habbena et al., 2009; Nicol et al., 2008). AOB abundance also increased with increasing disturbance intensity which is in agreement with similar studies conducted at Coweeta (e.g. L. Lin et al., 2017). Unlike AOA and AOB, we only found the effect of hillslope significant for CAOB abundance which is in contradiction to similar studies that found that all three nitrifiers increased in disturbed forests (e.g. Osburn & Barrett, 2020). CAOB abundance increased down a hillslope gradient likely in response to vegetation controls on microbial abundances as well as abiotic soil conditions (e.g. moisture and pH) that are favorable (Osburn et al., 2019). One possible explanation for the lack of relationship between our other variables and CAOB is the apparent ubiquity of CAOB across our sites.

For denitrifiers, we also found partial support for our hypotheses. The abundance of denitrifiers has been well studied in agricultural systems (Philippot et al., 2007), yet the effect of forest land-use on denitrifiers is still poorly known. In our system, both *nirK* and *nirS* increased with increasing disturbance intensity likely because of the N fertilizer addition from the agricultural activities in our most disturbed watershed (Attard et al., 2011; Elliott et al., 2017). Both *nirK* and *nosZ* increased with increasing soil pH, which supports our hypothesis and coincides with the patterns of nitrifier abundance as well. Increasing soil pH in this system coincided with increased N pools and transformation rates which could lead to increased abundance of functional microbial groups such as denitrifiers. We also found that *nirS* and *nosZ*

abundances increased down a hillslope gradient. Since denitrification is performed by facultative anaerobes, the abundance increasing down a hillslope likely reflects the anoxic conditions created by increasing soil moisture towards the toe-slope.

Considerations

There are many difficulties of using long-term data to explain ecological processes. The most recent forest survey we used to calculate mycorrhizal dominance and cumulative SNF occurred ~ 10 years prior to our soil sampling. As a result, there could be shifts in mycorrhizal dominance and SNF that occurred within the last decade that we could not account for. However, the stands used in this study are 44-, 54-, and 94-years post-disturbance, a time when forest composition is relatively stable (Waide, 1988). We also know that SNF by black locust is concentrated within the first few decades following disturbance (Wurzburger et al., 2021), so it is unlikely that cumulative SNF has increased much over the last decade. Given that black locust was most abundant several decades ago, we used a statistical model to estimate SNF, parameterized by contemporary data collected at this forest site. However, we acknowledge that SNF was not directly measured in these plots. Additionally, the pH of the soils prior to disturbance in unknown, thus we are only able to comment on trends associated with soil pH, not attribute causality. Because the plots in this study were long-term vegetation plots, we were also limited by their placement. The clear-cut watershed only had four toe-slope and ridge plots, so it is not a balanced design. While there were two fewer plots in the clear-cut watershed, we were still able to take an average of data from four data points where applicable. We also removed one mid-slope plot from the selectively cut watershed because it was not surveyed in the most recent forest survey. The most recent forest stand data from that plot would be over two decades old.

Another limitation is our methodology for quantifying N pools and fluxes. While resin bags are commonly used as an estimate of nutrient bioavailability, they simulate a set nutrient uptake rate whereas the uptake rate of plants varies by species and even individuals. Therefore, the bioavailability rate of our resin extracted inorganic N should be taken as an approximate estimate rather than a true *in-situ* bioavailability.

We used piecewise structural equation models to infer relationships among our variables, and the linear equations were evaluated individually, which allows for the fitting of smaller data-sets. However, our datasets consisted of a sample size of 42 whereas we included six parameters in our structural equation models. While this ratio of parameters to sample size may be appropriate for the local estimation method used by structural equation models, it likely still limits the confidence of the conclusions we are able to draw from our models.

We omitted microbial abundance data where abundance could not be detected. We lacked AOA gene abundance data from four plots in the selectively cut watershed: two were in the ridge, one was in the mid-slope, and one was in the toe-slope. We lacked AOB gene abundance data from five plots in the selectively cut watershed and one in the clear-cut watershed: In the selectively cut watershed, three were in the ridge, one was in the mid-slope, and one was in the toe-slope. In the clear-cut watershed, one was missing in the mid-slope. We lacked *nirK* data from two ridge plots in the selectively-cut watershed. As a result of the missing data, we took the average abundance for each plot.

Conclusion

Our study presents a mechanism through which ecosystem processes, and particularly the N cycle, can be altered for decades following land-use disturbance. Our findings suggest that N fixers have the potential to create biogeochemical founder effects that influence trajectories of

ecosystem recovery. While SNF is generally considered an ecosystem recovery mechanism (Boring et al., 2014; Boring & Swank, 1984; Zheng et al., 2020), we found that high rates of SNF can alter the trajectory of forest recovery. Specifically, we found that more intense land-use disturbance led to increased SNF by black locust which led to greater AM dominance, representing a transition from ECM dominance historically. We found that this shift to AM dominance subsequently increased N pools, N transformation rates, and the abundances of N cycling genes either directly or indirectly through soil acid-base chemistry. This suggests increased reactive losses of N following disturbance long-term. This study emphasizes the importance of studying the long-term legacy effects of land-use holistically as we saw that increasing disturbance intensity altered ecosystem processes directly and indirectly over the course of decades. SNF is a prevalent source of N in most biomes (Steidinger et al., 2019). Therefore, it is important to consider the degree of disturbance and long-term ecosystem processes globally, especially in systems where alternative stable states are possible.

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