

RESEARCH ARTICLE

Evaluation of soil properties and bulk $\delta^{15}\text{N}$ to assess decadal changes in floodplain denitrification following restoration

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Stream and floodplain restoration is a popular billion-dollar industry in the United States, with many restorations being conducted to satisfy water pollution regulations and nutrient reduction goals. The long-term efficacy of these restorations is, however, not well studied, and key soil metrics that can be used for performance assessments have not been developed. We evaluated a chronosequence of 12 restoration sites spanning an age range of 0–22 years to assess changes in denitrification rates and associated soil parameters. Restored versus unrestored reaches were compared for denitrification rate and functional gene *nosZ*, bulk soil $\delta^{15}\text{N}$, soil organic carbon (SOC), soil organic matter (SOM), bulk density, and soil moisture. Denitrification, SOM, SOC, and soil moisture were all found to increase with site age at restored sites, with the largest increase for the 10–22 age category. Bulk density decreased with time, with a significant decrease in restored floodplain soils. Bulk soil $\delta^{15}\text{N}$ was highest immediately after restoration, decreased with restoration age, and was not positively correlated with denitrification. This may reduce its potential as a proxy for denitrification. Overall, this study reveals that selected soil metrics (SOC, SOM, soil moisture, and bulk density) could serve as a valuable proxy for denitrification and could help assess the denitrification effectiveness of floodplain restorations at the decadal time scales. Ideally, the soil metrics should be combined with other short-term assessment measures, such as those for stream and groundwaters, for a robust performance assessment of restored floodplains.

Key words: bulk density, chronosequence, floodplains, mid-Atlantic, organic carbon, soil health, stable nitrogen isotopes, stream restoration

Implications for Practice

- Soil characteristics have not been routinely used for assessing the long-term effectiveness of stream and floodplain restorations, and guidance is needed for their use.
- Soil organic matter, moisture, and bulk density improved over time for restored floodplains and could be used as reliable proxies for denitrification nitrogen removal.
- Site-specific differences in restoration design and construction (e.g. soil amendments such as woody debris/chips) could significantly affect the response of soil metrics.
- Since soil characteristics change slowly over decadal time scales, restoration assessments should also include quicker, short-term performance measures such as those associated with surface and groundwater quality.

Introduction

Anthropogenic activities such as agriculture and urbanization and their legacies have significantly altered the geomorphic structure, flow regime, and water quality of streams and rivers across the United States and worldwide (Galloway et al. 2004; Walter & Merritts 2008; Inamdar et al. 2021). These legacies have resulted in impaired streams with flashy flows, elevated

sediment and nutrient concentrations, and poor ecological habitat (Kaushal et al. 2008; Forshay et al. 2022; Mayer et al. 2022). Stream restorations seek to remedy these conditions by reconnecting stream channels with their floodplains, decreasing streamflow velocities, and enhancing infiltration, reducing peak discharges and sediment loads, and providing a healthy, carbon-rich soil environment for biogeochemical processes to remove excess nutrients (Wohl et al. 2015; Mayer et al. 2022;

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Inamdar et al. 2023). Stream restorations are accomplished through raising stream beds, introduction of channel meanders, pools, and riffles, removal and/or grading of streambank sediments, and establishment of native riparian or wetland revegetation on the restored floodplain (Wood & Schueler 2020).

Stream and floodplain restorations have especially become popular in the United States, with more than a billion dollars spent annually nationwide (Bernhardt et al. 2005) and 400 million being spent since 1990 in the Chesapeake Bay watershed alone (Hassett et al. 2005). Similarly, the number of stream restorations has grown exponentially every decade (Bernhardt et al. 2005), and the average restoration length has almost tripled, from 233 to 670 m (Dance 2020). Many of the streams targeted for restoration are listed on the state's total maximum daily load (TMDL) listing of impaired water bodies. Thus, sediment and nutrient improvements/gains from restoration are being used as credits to meet water quality regulatory (TMDL) targets (Berg 2014; Mattern et al. 2020).

While restoration efforts have provided important gains toward enhancing stream, floodplain, and ecosystem health, many questions and concerns remain about their long-term effectiveness for improving water quality (Bernhardt & Palmer 2011; Palmer et al. 2014; Dance 2020). Typically, restorations implement beneficial conditions for stream and floodplain hydrology, vegetation, and soil type with the implicit assumption that nutrient removal functions and benefits will follow automatically. In essence, the projects follow the “Field of Dreams” paradigm of “if you build it, they will come” (Sudduth et al. 2011). Whether this actually happens or not is not yet clear.

A key reason for the knowledge gap on effectiveness or performance of the restoration projects is that many of the stream restoration projects have limited monitoring component, pre- or post-restoration, which prevents knowing whether the restorations achieved their water quality objectives or not (Palmer et al. 2014). For example, Hassett et al. (2005) reported that only 5.4% of the 4700 projects in their synthesis of studies in the Chesapeake Bay watershed report monitoring. This lack of monitoring has been because of (Buchanan et al. 2014): (1) unavailability of funding and resources; (2) lack of regulatory requirements; (3) absence of reliable and robust metrics for assessment, particularly long term; and (4) natural variability in system response because of spatial and temporal (e.g. baseflow vs. stormflows) differences and climate/weather variations (Filoso & Palmer 2011; Williams et al. 2017). If we are to enhance the effectiveness and resilience of these restorations, increase the return on our large investments, and address stakeholder and media concerns (e.g. Cox, 2018; Dance 2020), we need to monitor the sites post-restoration and develop robust metrics to assess the recovery of important ecosystem services such as denitrification (Kaushal et al. 2023).

Denitrification is a key ecosystem service provided by stream and floodplain restoration that is central to nitrogen (N) removal (Kaushal et al. 2008; Vidon et al. 2010; Mayer et al. 2022). Denitrification converts reactive nitrate-N (NO_3^-) in runoff waters to inert N_2 gas and thus reduces N pollution (Zametske et al. 2012). Frequent inundation of floodplain soils via over-bank flooding and floodplain reconnection is beneficial for

denitrification as this provides the necessary soil moisture and anoxic environment for the process (Mulholland et al. 2009; Vidon et al. 2010; Roley et al. 2012). Similarly, increased organic carbon inputs from restored riparian vegetation provide the energy that enhances denitrification (Gift et al. 2008; Mayer et al. 2010; Newcomer et al. 2012).

Previous restoration monitoring studies have typically assessed short-term N concentration changes in surface and groundwaters, and only a few have measured denitrification rates and identified their key drivers in floodplain soils over decadal time scales (Newcomer Johnson et al. 2014; Forshay et al. 2022; Mayer et al. 2022). Napora et al. (2023) studying a 1 to 10+ year chronosequence of restored floodplains in the mid-Atlantic United States, found that total carbon and N in floodplain soils increased over time and attributed it to increased soil wetness and soil organic matter (SOM) content. Forshay et al. (2022), working in Pennsylvania, United States, found that floodplain soil denitrification increased over a 5-year period and suggested that increased accumulation of soil organic carbon (SOC) was a key driver. However, Forshay et al. (2022) and Peck et al. (2024) also noted that recovery of ecosystem processes like denitrification on restored floodplains could take time.

Similarly, bulk soil $\delta^{15}\text{N}$ has also been used as a proxy for microbial denitrification (Hasselquist et al. 2017; Roberts et al. 2023). $\delta^{15}\text{N}$ represents the ratio of heavier ^{15}N to lighter ^{14}N isotopes and can be used to provide an integrated response of multiple N processes in soils (Bedard-Haughn et al. 2003; Nestler et al. 2011). Fractionation processes either preferentially remove ^{14}N from the environment to enrich $\delta^{15}\text{N}$ or add ^{14}N to deplete $\delta^{15}\text{N}$. Denitrification has a strong enrichment effect on $\delta^{15}\text{N}$ due to preferential conversion of ^{14}N molecules to N_2 gas, while processes like nitrification deplete $\delta^{15}\text{N}$ via addition of ^{14}N (White et al. 2021). Studying a 25-year chronosequence of 17 restored sites along Boreal riparian floodplains in Sweden, Hasselquist et al. (2017) found that foliage and soil $\delta^{15}\text{N}$ initially increased after restoration but then declined thereafter to more stable values. They attributed the initial increase in $\delta^{15}\text{N}$ to denitrification enrichment, which itself was stimulated by the short-term pulse increase in N due to restoration-related soil disturbance and the associated mineralization and nitrification of N. The decline in $\delta^{15}\text{N}$ thereafter during the “reorganization” restoration phase was attributed to a decrease in microbial processing and a lack of N availability. Others have suggested that the soil and foliage post-restoration $\delta^{15}\text{N}$ response could be complex, as it is affected by multiple drivers, including inputs from various N sources (atmospheric deposition, fertilizers, manure, etc.), mycorrhizal fungi and their role in N uptake by plants, and changes in riparian vegetation species, particularly N fixers that can increase availability of ^{14}N (Hobbie & Quimette 2009; Bai et al. 2013). In comparison to bulk soil and foliage $\delta^{15}\text{N}$, many studies have investigated the $\delta^{15}\text{N}$ values for dissolved NO_3^- and have shown that riparian floodplains and soils are hotspots for denitrification and N removal (Kaushal et al. 2008; Sigler et al. 2022). While surface or ground water $\delta^{15}\text{N}$ values represent a short-term metric for denitrification effectiveness, soil and foliage $\delta^{15}\text{N}$ values could provide a longer term measure of denitrification effectiveness.

Our interest in this study was to assess the use of soil properties (e.g. bulk density, SOM, soil moisture, and total soil C and N pools), including $\delta^{15}\text{N}$, for denitrification N removal on floodplain soils over the long term. We investigated 12 floodplain sites with restored and unrestored reaches in the mid-Atlantic United States that spanned a chronosequence of 0–25 years. Key questions that we addressed were: How do denitrification, soil bulk $\delta^{15}\text{N}$ and associated soil properties change following restoration? How do the restored floodplain properties compare with unrestored sites? Are there specific soil parameters that are more sensitive and provide a robust proxy for denitrification post-restoration? Specific hypotheses associated with the questions were: H1: soil moisture, SOC, and denitrification rates will increase in floodplain soils following restoration. H2: Bulk density will initially increase (because of restoration related compaction) but then decrease over time at restored sites. H3: Bulk soil $\delta^{15}\text{N}$ will initially increase due to disturbance related pulse increase in N but then will decrease over time as the floodplain soils and vegetation stabilize and recover. These hypothesized changes and trajectories for denitrification and soil parameters are illustrated in the conceptual model in Figure 1. This study will provide important guidance on which soil metrics can be used to assess denitrification N removal functions following restoration and could be used as indicators for future restoration assessments (Inamdar et al. 2023).

Methods

Study Sites

Floodplain restoration sites that were sampled across Maryland and Virginia included—Broad Creek, Kelly Branch, Stoney Run, Gramies Run, Scotts Level Branch (at Marriottsville

Rd.), Scotts Level Branch (at McDonogh Rd.), Bacon Ridge, Difficult Run, Catonsville Park, Minebank Run, Spring Branch, and Howard's Branch (Fig. 2). All sites were located in the Piedmont region of the United States, and specific coordinates, dates of restoration, soil types, impervious surface cover, and drainage area for the sites are reported in Table 1 (StreamStats 2022; Web Soil Survey 2024). Sites were grouped into a chronosequence of four age categories 0–2, 2–5, 5–10, and 10–22 years since restoration, with three sites in each category (Table 1). These sites mainly used the Natural Channel Design (NCD) of restoration methodology. Restorations that had additional features included Broad Creek which used step pools in the more upstream sections of its restoration to more effectively reduce the velocity of stormflow, and Howard's Branch and Bacon Ridge, which extensively incorporated woody debris from construction into the project design. Gramies Run also had partial legacy sediment removal incorporated into its design (Mattern et al. 2020).

Field Sampling

Floodplain soil and stream water samples were collected from the sites between April and May, 2023. A shovel was used to remove brush, foliage, organic matter, and restoration fabric (if present) from the floodplain surface, and soil samples were collected within the top 20 cm of mineral soil depth and stored in labeled one-quart zip-lock bags. Ten soil samples were collected from each site. Six soil samples were collected from each restored reach, with three from each bank at 1, 2, and 3 m from the stream edge. Four samples were collected from the upstream unrestored reach at each site, with two samples from each bank at 1 and 3 m from the stream edge. The 2-m sample at unrestored sites was omitted as funding was limited and only available for

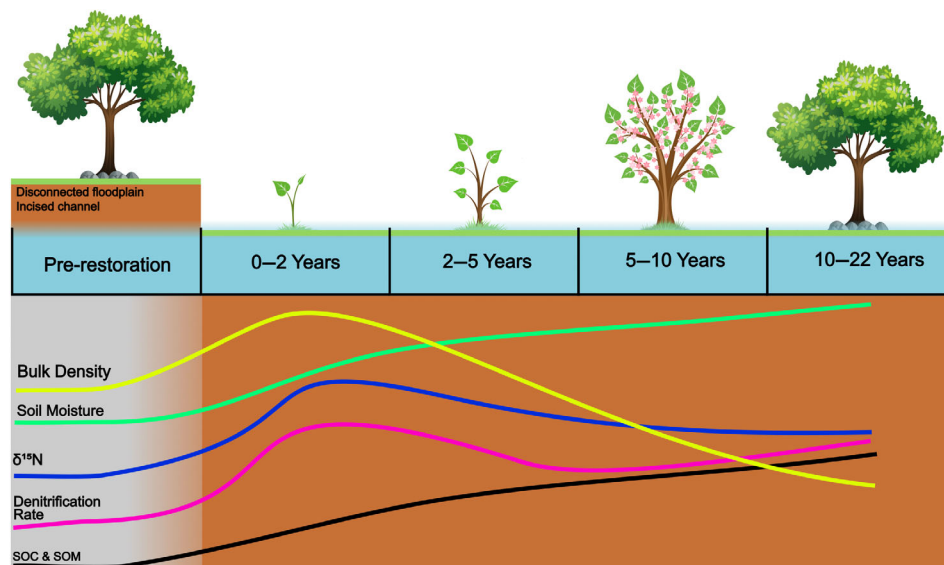


Figure 1. A conceptual diagram illustrating the hypothesized changes and trajectories of denitrification and associated soil properties (FREEPIK 2023; macrovector 2023). Soil moisture, SOC, and SOM are expected to increase after restoration as time progresses. Bulk density, $\delta^{15}\text{N}$, and denitrification are expected to initially increase due to restoration related disturbance and then decline to a relatively steady state. Bulk density is expected to decrease after initial compaction due to construction.

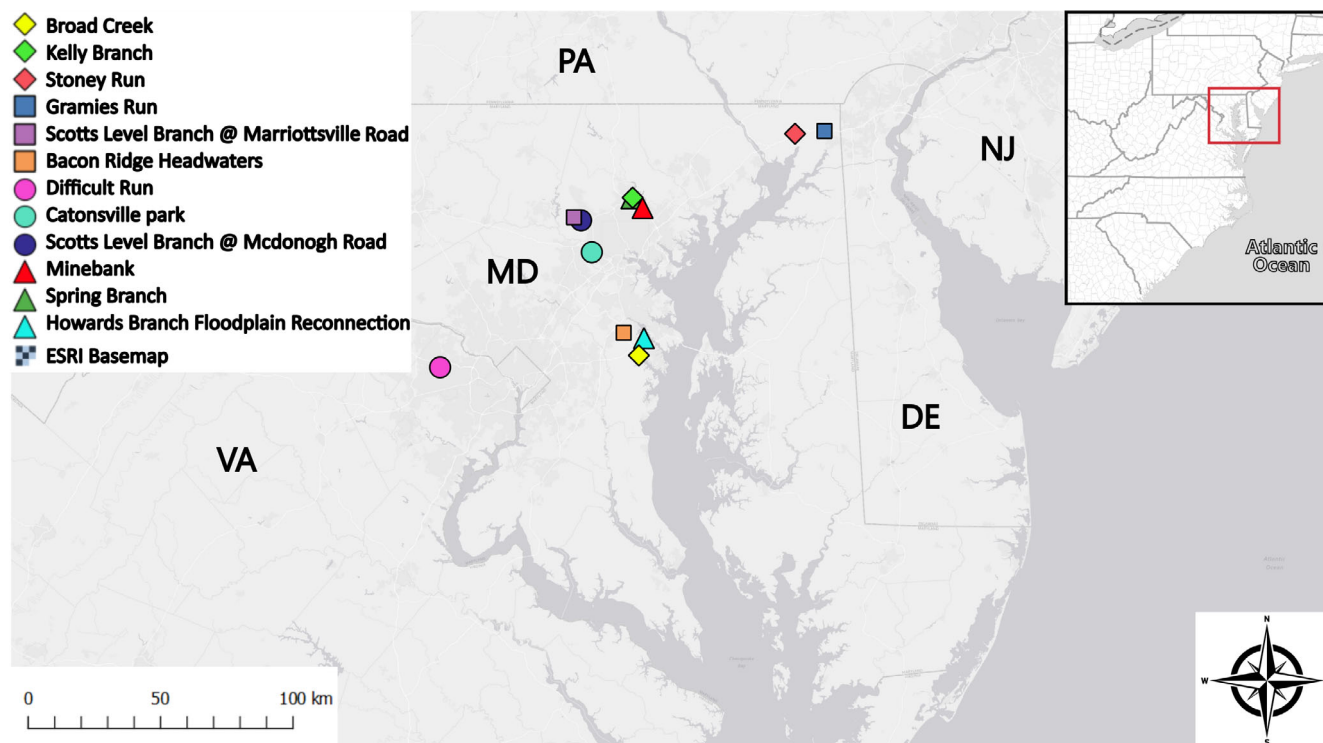


Figure 2. Locations of the 12 sampling sites selected for this study in Maryland and Virginia, United States. Sites are all within the Chesapeake Bay watershed and have had a floodplain restoration conducted within the past 22 years.

10 samples per site to be analyzed. Stream water samples were collected in acid-washed 250 mL Nalgene bottles from approximately the transition point between the unrestored and restored reach. Soil and water samples were transported in a cooler with ice and kept in a lab refrigerator.

Water samples were filtered and acidified with HCl to a pH of less than 2.

Soil bulk density was also determined for all restoration sites using aluminum cylinders with a known length and inner diameter. Sample collection was performed for mineral soil after the

Table 1. Descriptors for the chronosequence of 12 floodplain restoration sites selected for this study. Sites are divided into four separate age groups 0–2, 2–5, 5–10, and 10–22 years. The exact coordinates of the restoration are provided along with the state the restoration was conducted in, soil type, drainage area, and the watershed's impervious surface cover (StreamStats 2022; Web Soil Survey 2024).

Site	Age range (years)	Date restored	State	Latitude/longitude	Soil type	Drainage area (mi ²)	Impervious surface cover (%)
Broad Creek	0–2	2021	Maryland	38.969415°, –76.568360°	Collington, Wist, and Westphalia—sandy to fine sandy loam	0.1	15.7
Kelly Branch	0–2	2021	Maryland	39.442490°, –76.592562°	Comus silt loam	1.26	26.9
Stoney Run	0–2	2022	Maryland	39.633255°, –75.964033°	Beltsville silt loam	0.14	9.67
Gramies Run	2–5	2020	Maryland	39.686514°, –75.850506°	Hatboro silt loam	1.26	4.03
Scotts Level Branch at Marriottsville Road	2–5	2019	Maryland	39.383717°, –76.819942°	Baile silt loam	0.25	56.4
Bacon Ridge	2–5	2020	Maryland	39.037240°, –76.627585°	Annapolis fine sandy loam	0.3	7.4
Difficult Run	5–10	2014	Virginia	38.933449°, –77.338004°	Wheaton Glenelg complex—silt loam	0.0544	32.3
Catonsville Park	5–10	2017	Maryland	39.279201°, –76.750729°	Watchung silt loam	0.0517	41.4
Scotts Level Branch at McDonogh Road	5–10	2014	Maryland	39.374473°, –76.792333°	Hatboro silt loam	1.42	36.9
Minebank Run	10–22	2005	Maryland	39.416075°, –76.547572°	Lindside silt loam	2.93	32.5
Spring Branch	10–22	2008	Maryland	39.440247°, –76.597526°	Melvin silt loam	1.46	34.3
Howard's Branch	10–22	2001	Maryland	39.021048°, –76.548883°	Annapolis fine sandy loam	0.36	12.1

top organic layer had been cleared by gently tapping the aluminum cylinder into the soil using a shovel. Excess soil was removed from the sample cylinder using a pocketknife, and the sample was stored in a sealed zip-lock bag for analysis in the laboratory.

Sample Analysis

Bulk density samples were dried at 65°C for 48 hours in a lab oven, and the dry mass of the soil was divided by the cylinder volume to get the density values. Soil porosity was calculated using the formula $((1 - [\text{bulk density}/\text{particle density}]) \times 100)$, where particle density was assumed to be 2.65 g/cm³. Gravimetric water content (GWC) was calculated by dividing the water mass by the soil mass of each sample.

SOM, SOC, and total nitrogen (TN) content were determined by Regen Ag Lab, Nebraska, following standard methods. SOM was determined via loss on ignition in a 400°C furnace. SOC and TN were determined via combustion. A Lachat instrument was used to determine KCl extracted NO_3^- and ammonium-N (NH_4^+) concentrations present in soil samples. Soil organic nitrogen (SON) was determined by subtraction of NO_3^- and NH_4^+ from soil TN.

Unamended denitrification enzyme activity (DEA) assays for the soil samples were conducted using gas chromatographic analysis to measure N_2O gas concentrations. The purpose of unamended DEA (hereafter referred to as denitrification) was to measure the expression of denitrification enzymes that are present in soil or sediment at the time of collection. Sample analysis followed methodologies described previously (Peck et al. 2023).

Bulk soil $\delta^{15}\text{N}$ analysis was determined at the University of Maryland Eastern Shore (UMCES) laboratory. An Elemental Analyzer and Isotopic Ratio Mass Spectrometer (IRMS) were used for sample analysis. Stream water nitrate $\delta^{15}\text{NO}_3^-$ concentrations were determined at the University of Nebraska Isotope Laboratory by the titanium trichloride reduction method (Altabet et al. 2019).

Nitrous oxide reductase (*nosZ*) functional genes that represent the microbial abundance and potential for denitrification (Kan et al. 2023) were determined at the Stroud Water Research Center using a subset of bulk soil samples (2 from the restored section of each site for a total of 24 samples). Detailed sample analysis and data processing were described previously (Peck et al. 2023).

Statistical Methods

To account for site-specific variation in soil properties and processes, we used linear mixed-effects (LME) to evaluate the effects of restoration status and age category on soil parameters (soil denitrification rate, SOM, SOC, bulk density, soil moisture, and $\delta^{15}\text{N}$). Each of these variables was individually assessed using a LME model, including site (three sites per age category) as a random effect and restoration status, age category, and their interaction as fixed effects. All dependent variables were log-transformed prior to the analysis. Error structures were applied to help normalize variance in the residuals due to heteroscedasticity observed among sites, but they did not significantly improve the LME model fit (i.e. reduced AIC) and they were

therefore removed. All LME models were fitted using restricted log-likelihood with the *lme* function in the package “nlme” in R (Pinheiro et al. 2018) and following the protocols described by (Zuur et al. 2009) to identify the optimal LME model. Normality and homoscedasticity results are shown in Tables S1 and S2, respectively.

We computed least-squares means for each fixed factor in the LME models using the “lsmeans” package in R to determine pair-wise comparisons among the means of respective independent variables and interactions among treatments to determine which age category and restoration status or combination thereof significantly influenced dependent variables. Spearman correlation and principal component analysis (PCA) were used to determine the relationships between denitrification rates and soil metrics, including soil moisture, bulk density, GWC, SOM, SOC, NH_4^+ , NO_3^- , TN, SON, and $\delta^{15}\text{N}$. Correlations with *p* values less than 0.05 and PCA vectors less than 90° are considered significant. Stream water nitrate $\delta^{15}\text{N}$ was also compared to bulk soil $\delta^{15}\text{N}$ to determine if changes in soil $\delta^{15}\text{N}$ were related to stream water nitrate $\delta^{15}\text{N}$.

Results

Differences in Denitrification and Soil Metrics Between Unrestored and Restored Sites and Changes With Time (Age Categories)

Although visually there was a clear difference in the temporal trends between age categories in restored and unrestored reaches (Fig. 3), the statistically significant differences (considering restoration status and age category) were limited to only SOC, SOM, and $\delta^{15}\text{N}$ (Table S3; Fig. 3A–C). SOC, SOM, and bulk density also showed significant differences between restored and unrestored reaches, and denitrification rate was found to vary significantly when only age category was considered (Table S3; Fig. 3A, 3B, & 3D). While multiple variables revealed increasing (denitrification, SOM, SOC, and SON) or decreasing (bulk density and $\delta^{15}\text{N}$) trends for the restored reaches with time (age categories), and there were some differences between restored and unrestored parameters (Table S3; Fig. 3), only a few had statistically significant pairwise differences. In the 10–22 years age category, both SOC and SOM had higher concentrations in restored floodplain soils than unrestored ($p = 0.0013$ and 0.0066 , respectively). $\delta^{15}\text{N}$ was also significantly higher in 0- to 2-year-old restored sites than in unrestored catchments of the same age ($p = 0.0443$). Finally, denitrification was found to be significantly higher at the oldest restored catchments sampled (10–22 years old) when compared to the youngest restored and unrestored soils (0–2 years age category) ($p = 0.0331$ and 0.0426 , respectively). While SON and soil moisture followed the same general increasing trend for restored sites with age, no significant differences were observed. Bulk density decreased with time for the restored sites, but temporal differences were not statistically different. Soil NO_3^- and NH_4^+ appeared to be the least sensitive parameters to temporal change and were the only soil parameters that did not have any discernible trend between restored and restored sites

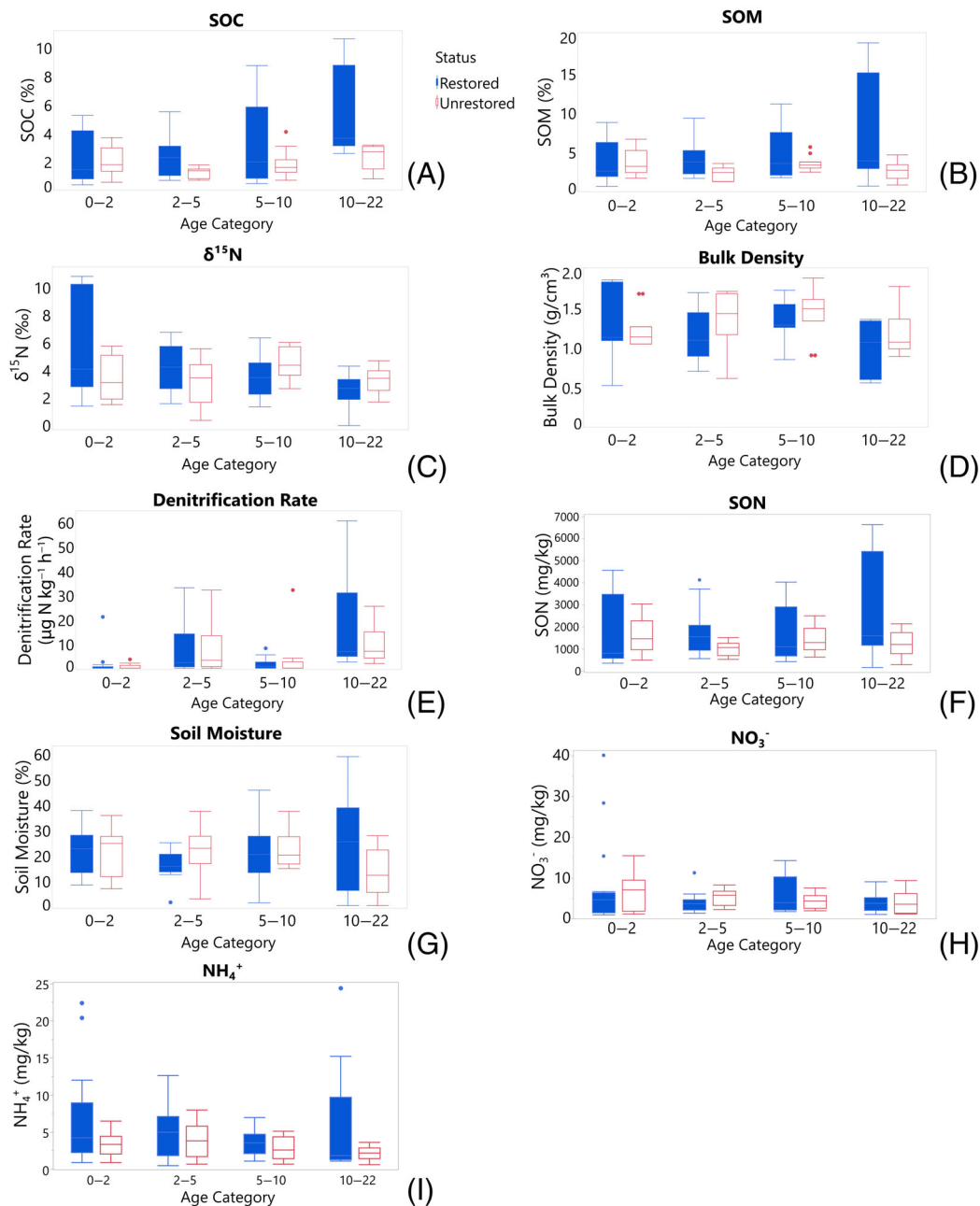


Figure 3. Box and whisker plots for various soil metrics by age category for restored (solid blue) and unrestored (hollow red) sites. These include: SOC (A), SOM (B), $\delta^{15}\text{N}$ (C), bulk density (D), soil denitrification rate (E), SON (F) soil moisture (G), NO_3^- (H), and NH_4^+ (I). Boxes show the first and third quartile, center lines are medians, whiskers indicate data minimums and maximums, and points outside the whiskers are outliers.

(Fig. 3H & 3I) with no statistical differences. Substantial variation between sites was found, with site-specific effects explaining most of the observed variance for all factors except for denitrification (Table S3). Mean and standard deviations for the individual sites within each age category are reported in Table S4.

Denitrification gene *nosZ* did not replicate the significant increase for denitrification in the 10–22 years age category and

was not significantly related to denitrification rates (Table S5; Fig. 4). PCA comparisons (Fig. 5) of soil parameters for individual sites (restored and unrestored reaches) highlighted the influence of site-specific features that drove the differences between the age categories. PCA for the restored sites revealed Stoney Run (0–2 age category) and Howard's Branch (10–22 age category) as distinct clusters in the right quadrants (Fig. 5), but notably, the same pattern was not replicated in the PCA for the

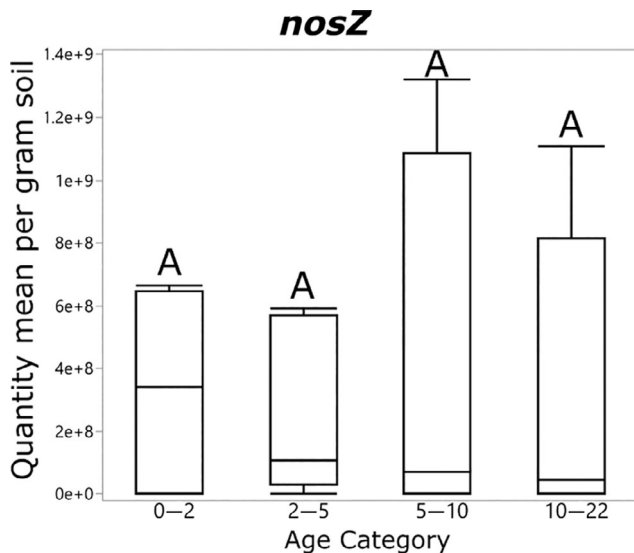


Figure 4. Box and whisker plots for *nosZ* abundance in restored floodplain soils. Significantly different results (as determined through one-way ANOVA and Tukey–Kramer HSD connecting letters) are included; different letters indicate significant differences. ANOVA, analysis of variance; AIC, akaike information criterion; ESRI, environmental systems research institute; HSD, honestly significantly difference.

unrestored sites. Restored soils at Stoney Run had significantly higher $\delta^{15}\text{N}$ than any other site (Table S6), and restored soils at Howard’s Branch had significantly higher SOM and SOC than any other site (Tables S7 & S8).

Relationships Between Soil Metrics and Denitrification Rates

The highest floodplain SOC concentrations are approximately two times higher at restored sites compared to unrestored reaches (Fig. 6). As SOC increases at restored sites, the relationship between SOC and NO_3^- shifts from positive (years 0–2, 2–5, and 5–10) to negative (years 10–22) (Table S9; Fig. 6). In comparison, at unrestored sites, the relationship always remained positive with similar positive linear slopes (Table S9; Fig. 6). Similarly, as GWC increased at restored sites past an inflection point (approximately 55%), its relationship with denitrification rate changed from negative to positive (Fig. 7). The negative trend was present up to 55% GWC at both restored and unrestored sites, with only restored sites having values above that threshold. Floodplain soils with GWC greater than 55% had significantly higher denitrification than soils with GWC less than 55% (Table S10).

Denitrification was positively correlated with SOC, SOM, TN, and SON, with bulk density being negatively correlated (Table S11). However, soil $\delta^{15}\text{N}$ was only positively correlated with NO_3^- and did not co-vary with denitrification (Table S11). PCA analysis (Fig. 5) also found that denitrification was clustered together with SOC, SOM, TN, and SON at restored sites, with no such observations for the unrestored sites. Stream water nitrate $\delta^{15}\text{N}$ did not reveal any significant relationships with bulk soil $\delta^{15}\text{N}$ (Fig. S1).

Discussion

Our data revealed that some soil metrics (SOM, moisture, and bulk density) can reveal important differences between restored and unrestored sites, can help characterize the long-term temporal evolution of floodplains post-restoration, and can serve as a good proxy for denitrification N removal function in restored floodplains. On the other hand, soil metrics like concentrations of NO_3^- and NH_4^+ , functional genes, and bulk soil $\delta^{15}\text{N}$ may not be sensitive and/or representative to characterize the changes in floodplain restorations or may be impacted by other parameters in addition to restoration age. Our results also reveal that large variations in soil properties (some associated with site-specific restoration strategies) could complicate the chronosequence comparisons of restorations across age categories. We elaborate on these observations in the discussion below.

Changes in Denitrification and Relationships With Soil Properties Following Restoration

Increased floodplain denitrification and associated N removal is one of the main objectives of stream and floodplain restorations (Kaushal et al. 2008; Mayer et al. 2022). Among our 12 restored sites, the denitrification rate was found to increase with time, with the oldest restorations (10–22 years age category) revealing the highest values. Variability in the denitrification dataset increased during the 2–5 years age period, possibly indicating that some “biogeochemical hotspots” were sampled at those restorations (Vidon et al. 2010). Other variables that significantly increased at restored sites over time included SOC and SOM, with soil moisture also increasing but not significantly. SOC and SOM were positively and significantly correlated with denitrification rate. This suggests that floodplain restorations are indeed improving these soil properties over decadal time scales, with subsequent improvement in denitrification ecosystem services. Increased bioreactive surface area through floodplain expansion, restoration, reconnection, and inundation can enhance NO_3^- removal through enhanced denitrification (Roley et al. 2012; McMillan et al. 2014; Speir et al. 2020). Increases to hydraulic conductivity as well as hydraulic residence time were also found to increase denitrification rates (Kaushal et al. 2008).

Denitrification is further enhanced during the course of over-bank flooding and inundation, especially in vegetated floodplain soils, which were found to have 107 times higher denitrification than identical control samples at ambient soil moisture (Roley et al. 2012; Yao et al. 2022). Organic carbon is the key energy source for heterotrophic microbes that drive denitrification (Vidon et al. 2010). Thus, established vegetation and associated SOM likely contributed to the elevated denitrification rates observed for restored sites in our 10–22 years age category.

Other potential sources of floodplain SOC and SOM could be more site specific, as some restorations intentionally incorporate woodchips and other woody debris from construction into their floodplain. In our study, the restored floodplain at Howard’s Branch was of note as it followed this approach and had significantly higher SOM and SOC than any other restored or unrestored site. McMillan et al. (2014) studying restored urban

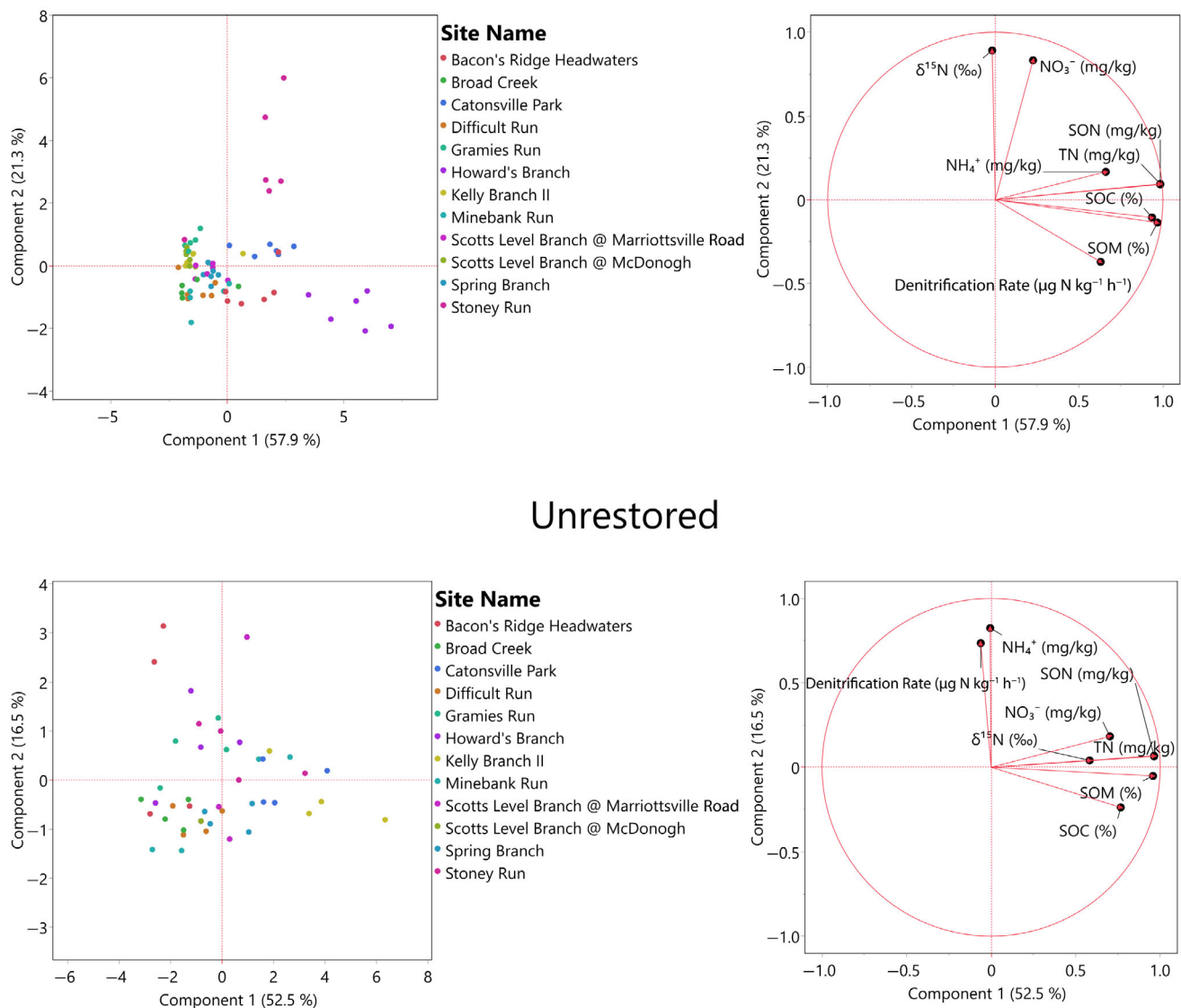


Figure 5. Principal component analysis (PCA) for unrestored and restored soil parameters by site. There is greater separation between select sites post-restoration. Stoney Run and Howard's Branch are notable outliers post-restoration.

streams in North Carolina also found that NO_3^- uptake was highest in older restored sites, likely due to increased channel stability and microbial community recovery. The results of this study are also similar to those reported by Napora et al. (2023), for a 1 to 10+ year chronosequence of sites in the mid-Atlantic United States region with one of their sites—Difficult Run—in our own data set. Napora et al. (2023) found that soil moisture, SOM, SOC, and TN increased with restoration age. They also reported that restored floodplains retained more SOC and TN than unrestored sites and also exceeded reference floodplains chosen for their low impervious surface cover watersheds (Napora et al. 2023).

Increases in SOM and SOC at our sites indicate that vegetation recovery after restoration is progressing well and post-restoration vegetation growth is adding more SOM to the

floodplain. Some of the increased SOC may also be originating from root decomposition of trees felled during restoration or intentional addition of woody debris to the floodplain and channel (Wood et al. 2022; Biohabitats 2024). Increased accumulation and processing of carbon can strongly regulate N dynamics in streams, and both riparian and wetland leaf litter is a major source of organic carbon and matter (Mayer et al. 2022). As a result of significant organic material accumulation, denitrification can sometimes be NO_3^- limited in floodplains and debris dams (Groffman et al. 2005; Kaushal et al. 2008; Taylor & Townsend 2010). This NO_3^- limitation due to increasing denitrification driven by increasing SOC is nicely illustrated where the slopes of the SOC— NO_3^- relationship shift from positive to negative values for the highest age category. As NO_3^- is quite mobile, we recognize that other site-specific factors may be influencing the decreasing trend

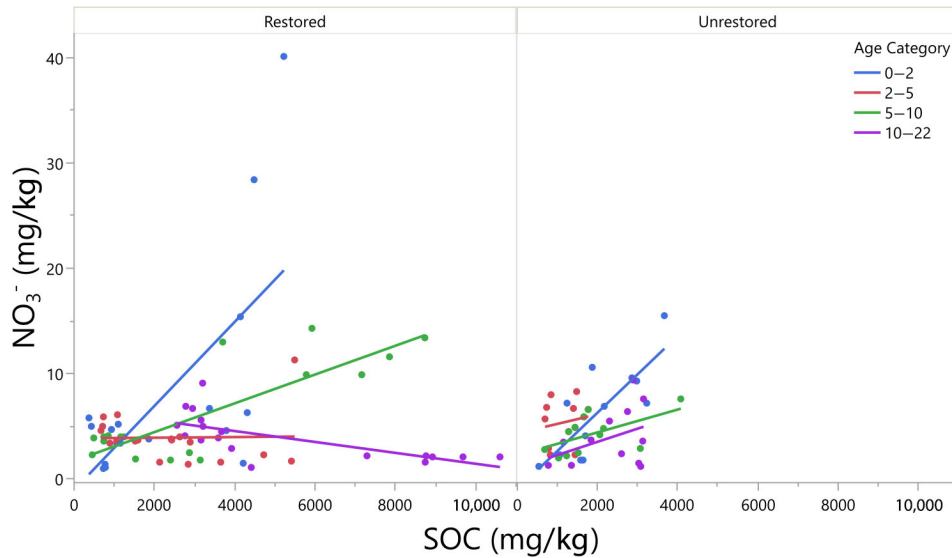


Figure 6. The changing relationship between NO_3^- (mg/kg) and SOC (mg/kg) between restored and unrestored floodplains. As SOC increases at restored sites, environmental conditions for denitrifying bacteria improve and NO_3^- concentrations decrease. In contrast, in unrestored catchments, the relationship between SOC and NO_3^- trends positively among all age categories.

observed. Similar trends were observed in other studies, however, with soil NO_3^- and SOC being correlated with denitrification (Yao et al. 2022).

Denitrification functional *nosZ* did not follow the temporal trend displayed by denitrification rates across the restoration age categories and were not correlated with the denitrification rates. This is not necessarily surprising since previous studies have also reported mixed results for correlations between functional genes and denitrification rates (Li et al. 2020; Ye et al. 2023). High variability in the results and the reduced

sample set used for these variables (24 out of 120 total) could have likely contributed to this inconclusive result. This suggests that *nosZ* functional genes may not necessarily be representative of the denitrification occurrence and/or soil environmental conditions were driving the floodplain denitrification rates.

In addition to increased SOM and SOC, moist and reducing soil conditions are also necessary for denitrification (Tan et al. 2018). The regrading and widened floodplain created during stream restoration increased soil moisture significantly and had the potential to increase groundwater–surface water interactions and hydrologic residence times (Kaushal et al. 2008). Increased hyporheic exchange combined with the increased SOM creates an ideal environment necessary for denitrification (Tan et al. 2018; Mayer et al. 2022). Optimal conditions for the highest rates of denitrification, however, occur in sediment, which is low in oxygen, high in organic carbon, and high in NO_3^- (Kaden et al. 2021). Over time, denitrification can diminish soil NO_3^- concentrations in organic rich, periodically inundated soils (Orr et al. 2007). This was observed in our results as well, with GWC also found to significantly co-vary with denitrification at restored sites in Spearman correlation and PCA results. Previous studies observed no denitrification when GWC was below 11%, with denitrification starting at concentrations of 18% (Hénault & Germon 2000). The threshold for pronounced denitrification in our data falls begins at a higher value of approximately 55%. The maximum GWC of restored sites ranges far higher (158%) than that found in unrestored reaches (54%), with accompanying significantly increased denitrification. Increasing organic carbon, denitrification, and soil moisture indicate that the oldest category of stream restorations studied provided optimal denitrifying conditions, including inundation of the soil via overbank flow (Kaden et al. 2021).

Bulk density was highest for the 0–2 age category, declined through time at restored sites, and was lowest for the 10–22

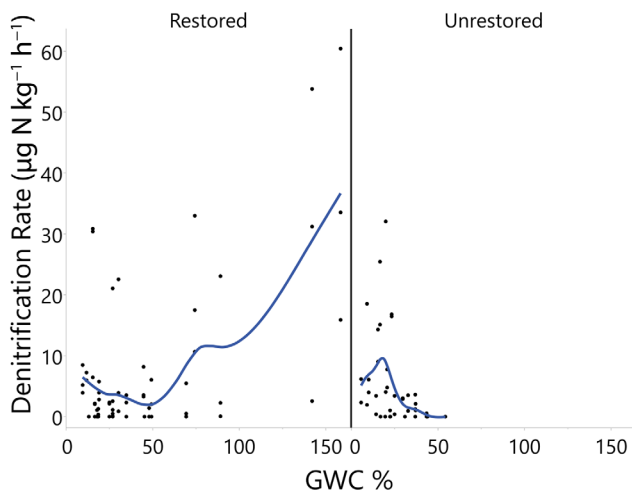


Figure 7. The effect of a more reconnected floodplain and ephemeral anoxic environment on floodplain soil denitrification. As gravimetric water content (GWC) increases, there is also an increase in denitrification rate at restored sites. Maximum GWC was also found to be approximately 3× higher at restored sites than unrestored sites. The blue line represents a moving average of results.

age category. The highest values of bulk density immediately post-restoration for the 0–2 age category are not surprising given that previous studies have indicated that restoration/construction activities result in compaction of floodplain soils (e.g. Laub et al. 2013). Importantly, our data revealed a significant negative correlation between bulk density and denitrification rates. Decreasing bulk density increases soil pore space, which in turn increases soil moisture retention and provides a favorable environment for microbes—all conditions that enhance denitrification.

Changes in Bulk Soil $\delta^{15}\text{N}$ With Restoration Age and Its Relationship With Denitrification

Soil $\delta^{15}\text{N}$ values for our restored floodplains were highest for the 0–2 years age category and then declined thereafter. Within the 0–2 age category, the $\delta^{15}\text{N}$ values were particularly elevated for the Stoney Run restoration site, which also had very high concentrations of soil and water NO_3^- . We speculated that the sharp increase in $\delta^{15}\text{N}$ in the 0–2 age category was likely due to increased denitrification losses stemming from—(1) immediate inorganic N availability from soil and site disturbance; (2) enhanced hydrologic connection with the stream immediately after restoration; and (3) plant uptake and fractionation (Kaushal et al. 2008). Our denitrification rates for the 0–2 age category, however, did not reveal the potential for elevated denitrification. A similar response for $\delta^{15}\text{N}$ was reported by Hasselquist et al. (2017), who found a sharp increase in foliage $\delta^{15}\text{N}$ immediately following restoration with a decline thereafter. This response (increase pulse increase in $\delta^{15}\text{N}$ followed by a long-term decrease) follows the model hypothesized by Molles Jr et al. (1998), where immediately after restoration, N processing is stimulated because of site disturbance. Subsequently, as the site reorganization progresses, $\delta^{15}\text{N}$ will likely decrease with time as N availability decreases and the N-cycle restabilizes (Molles Jr et al. 1998; Hasselquist et al. 2017). Our observations for $\delta^{15}\text{N}$ follow the decline hypothesized, the conceptual model of Molles Jr et al. (1998), and observations reported by Hasselquist et al. (2017).

Other potential drivers of the steady decline in $\delta^{15}\text{N}$ for restored sites could be due to increased vegetative growth, which is often a major objective of stream restoration (Palmer et al. 2014). With increased vegetative biomass, there is organic N accumulation in the floodplain soil (Michener & Lajtha 2007). SON in our dataset did increase at restored sites over time but not significantly. As organic N mineralizes, NH_4^+ increases the ^{14}N fraction and depletes the $\delta^{15}\text{N}$ in the soil (Michener & Lajtha 2007). This fractionation can also be influenced by the rate of depolymerization of amino acids in soil SOM (which occurs locally in microsites in the soil matrix) (Schimel & Bennett 2004). Because of this and due to soil N being predominantly in a large, non-reactive pool, bulk soil measurements may not provide adequate information on the $\delta^{15}\text{N}$ of N, which has been metabolized by organisms (Michener & Lajtha 2007; Hobbie & Ouimette 2009). Differences in slope position, topography, and drainage may also significantly influence the $\delta^{15}\text{N}$ present in samples collected, as can the spatial variability of

organic N mineralization (Bedard-Haughn et al. 2003; Bai et al. 2013). Contamination with animal waste or sewage, which possess an enriched/elevated $\delta^{15}\text{N}$ signature, could also affect individual soil samples (Bedard-Haughn et al. 2003; Bai et al. 2013). Sewage contamination may have been a possibility at Stoney Run as the restoration also addressed sewage leakage issues, but the enriched signature was not observed in the stream water nitrate $\delta^{15}\text{N}$ value.

The change in floodplain plant species composition could also affect the bulk soil $\delta^{15}\text{N}$. Stream and floodplain restoration projects typically result in substantial disturbance and/or removal of existing floodplain vegetation, especially for sites where legacy sediment is excavated (Mattern et al. 2020). Restorations also tend to increase colonization by non-native plant species that previously may not be present (Hunter & DeBerry 2023). Introduction of new vegetation could lead to depletion in bulk soil $\delta^{15}\text{N}$. In particular, N_2 fixing species like clover and Honey mesquite (*Prosopis glandulosa*) tend to introduce N into the soil through symbiotic fixation with a $\delta^{15}\text{N}$ at or near zero, resulting in further depletion of floodplain soil $\delta^{15}\text{N}$ (Boeckx et al. 2006; Bai et al. 2013; Irisarri et al. 2019).

This study showed that select soil metrics can be used successfully to characterize differences between restored and unrestored reaches and the decadal-scale temporal changes in restored reaches over time, particularly with regard to denitrification. For assessing denitrification changes post-restoration, SOC, SOM, SON, and soil bulk density were the most effective, sensitive, and reliable soil metrics. SOM and SOC increased with restoration age, while bulk density displayed a decreasing trend. On the other hand, concentrations of NH_4^+ , NO_3^- were least useful in quantifying denitrification changes post-restoration, though site-specific factors could have caused this effect. Our work also suggests that bulk soil $\delta^{15}\text{N}$ may be influenced by a number of factors, particularly new floodplain vegetative growth, and was not a robust metric to characterize soil denitrification trends over the long term.

This study also highlighted that soil denitrification and associated soil metrics may take time to reflect the improvement in floodplain health associated with restoration. In contrast, studies that have assessed denitrification in ground and stream waters have found quicker restoration responses (e.g. Kaushal et al. 2008; McMillan et al. 2014; Newcomer Johnson et al. 2014; Forshay et al. 2022), indicating differential time lags across various ecosystem compartments. Thus, ideally, a combination of response metrics spanning different time scales across water and soil components should be implemented to assess long-term stream and floodplain restoration changes. Surface water measurements could be used to assess short-term (0–5 years) denitrification changes, while soil metrics could be used to capture the integrated, decadal scale changes in restoration.

We should also recognize that chronosequence studies that include multiple restoration sites (like ours) could be affected by shifts in soil metrics associated with site-specific restoration strategies (e.g. the use of wood chips at the Howard's Run site) and could complicate the statistical comparisons across time. Unfortunately, this is an inherent challenge of chronosequence

studies and can be overcome by increasing the number of sampled restoration sites to help reduce the significance of site-specific effects.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. QQ plot and AICc results used to determine a variables' normality.

Table S2. Levene's test, used to determine a variables' homoscedasticity.

Table S3. Mixed-effects model results for bulk density, SOC, SOM, $\delta^{15}\text{N}$, soil moisture, denitrification rate, SON, NO_3^+ , and NH_4^+ .

Table S4. Median and standard deviation of data used in Figure 3 (A–I) and Table S3. Data are organized by site name and age category.

Table S5. Spearman Correlation results for denitrification functional genes *nosZ*.

Table S6. $\delta^{15}\text{N}$ by site and restoration status ANOVA. Different letters represent a statistically significant difference.

Table S7. SOM 2-way ANOVA by site and restoration status.

Table S8. SOC 2-way ANOVA by site and restoration status.

Table S9. Linear trends between NO_3^+ (mg/kg) and SOC (mg/kg) shown in Figure 5.

Table S10. ANOVA for GWC and denitrification.

Table S11. Spearman's rank correlation coefficients color coded from green (highest) to red (lowest).

Figure S1. Linear regression of stream nitrate versus soil $\delta^{15}\text{N}$ and stream nitrate $\delta^{15}\text{N}$ versus bulk soil $\delta^{15}\text{N}$.

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