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Evaluation of the effectiveness of riparian zone restoration in the southern Appalachians by assessing soil microbial populations

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Abstract

Microbial biomass, nitrifiers and denitrifiers in surface soil (0–10 cm) were quantified in a riparian zone restoration project at Coweeta, North Carolina, USA. Four treatments are included in this study: (1) a degraded (+N) riparian zone with continued compaction, vegetation removal, and nutrient addition (mow, roll, and nutrient addition); (2) a degraded (−N) riparian zone with continued compaction and vegetation removal, but without nutrient addition (mow and roll only); (3) a restored riparian area (no grazing, cessation of manure and N fertilizer application and re-vegetation with natural regrowth for 2 years); and (4) reference riparian zone (no riparian zone degradation has occurred within the last 10 years). Mean microbial biomass C increased from winter (316 mg kg^{-1}) to summer (593 mg kg^{-1}), and decreased from summer to fall (265 mg kg^{-1}). There were no significant changes in microbial biomass C with the cessation of manure and chemical fertilizer application in the degraded − N plot and with re-vegetation in the restored plot as compared to the degraded + N plot. Microbial biomass C level in the restored plot was comparable to that in the reference plot for most seasons. Restored plots had significant greater populations of denitrifiers than reference plots in the spring. Nitrifier numbers were lower in the degraded − N and restored plots than the degraded + N. Ammonium oxidizers in summer were more abundant (25,000 g^{-1} soil) in the degraded + N plot compared to (1000 g^{-1} soil) in the degraded − N and restored plots, and NO_2^- oxidizers in the same period were more abundant (130,000 g^{-1} soil) in the degraded + N plot than that in the degraded − N and restored plots about (40,000 g^{-1} soil). The soil NO_3^- concentrations were considerably lower in the degraded − N and restored plots than the degraded + N plot. Our results imply either cessation of manure and N fertilizer application or cessation of manure and N fertilizer and re-vegetation could contribute to restoration of degraded riparian zone through reducing numbers of nitrifiers.
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1. Introduction

Degradation of riparian zones is considered as a major contributor to non-point source nutrient inputs to streams throughout the eastern USA. This

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degradation is primarily the result of farming and livestock grazing activities that compact soil and reduce vegetation cover. In western North Carolina, concerns center around maintaining drinking water supplies for local communities and providing aquatic and terrestrial wildlife habitat. Restoration of degraded riparian zones is currently being conducted by teams of local, state, and federal entities throughout the region. Research is needed to examine rates of recovery and overall effectiveness across a range of restoration alternatives.

We hypothesized that one of the benefits of riparian restoration would be an increase in microbial biomass. This increase would subsequently play an important role in reducing N inputs to the stream, as microbial biomass plays an important role in preventing N loss (Henrot and Robertson, 1994). In recovering grazed and pastured systems, many mechanisms influence microbial biomass. For example, while increased C input and reduced compaction under re-vegetation restoration may result in increased microbial biomass (Witter and Kanal, 1998), eliminating manure inputs may have a negative effect (Acea and Carballas, 1988; Ritz et al., 1997). In contrast, reducing inputs from chemical fertilizer could be beneficial to microorganisms in riparian zones, according to Ettema et al. (1999) who reported that addition of N fertilizer reduced microbial biomass C in a riparian forest of the coastal plain of Georgia, USA.

Nitrifiers and denitrifiers have a strong influence on the concentration of NO_3^- in riparian systems. Denitrifiers transform NO_3^- to other forms of N, whereas nitrifiers produce NO_3^- by oxidizing NH_4^+ and NO_2^-. It is reasonable to assume that increase in denitrifiers and decrease in nitrifiers would lead to a reduction of NO_3^- in riparian systems. Management practices, which favor denitrifiers and inhibit nitrifiers, while maintaining a high microbial biomass, should be considered promising for the riparian restoration. The flux of trace gases, e.g. N_2O and NO, along with land use change and soil management has received increased attention (Sanhueza, 1997) because nitrous oxide contributes to stratospheric ozone destruction and nitric oxide is the limiting precursor to tropospheric ozone production. The production of nitrous oxide (N_2O) is the result of denitrification (Schipper et al., 1993), while nitrification is the main source of soil NO (Anderson and Levine, 1986). Quantifying population of nitrifiers and denitrifiers should be useful for modeling impacts of the proposed restoration on trace gas emission from riparian soils.

In this study we examine the effects of riparian zone restoration on total microbial biomass, denitrifying bacteria, and nitrifying bacteria to evaluate the effectiveness of such restoration in reducing NO_3^- input to stream systems and trace gas emission to atmosphere.

2. Materials and method

2.1. The study site and sample collection

The study was conducted on the Slagle farm located in SW North Carolina. Prior to fencing to exclude cattle in 2000, heavy and continuous cattle use resulted in areas of significant soil compaction, reduced vegetative cover, and direct nutrient inputs from cattle waste. In June 2000, a 10 m riparian buffer was established by fencing to exclude cattle. The riparian area borders Cartoogechaye Creek, a tributary of the Little Tennessee River. Elevation is 670 m above sea level (asl). The climate is classified as Marine and Humid with cool summers and mild winters with a mean annual temperature of 13 °C and annual precipitation of 1780 mm. The soil is slightly acidic.

After fencing, physical and chemical impacts of grazing within the riparian zone were simulated by experimentally controlled compaction, grazing, and nutrient additions. This experimental approach was implemented to reduce spatial heterogeneity which might preclude detection of changes in soil pools and processes. The experiment was laid out as a randomized complete block design with four replications of the following treatments: (1) a degraded (+N) riparian zone with continued compaction, vegetation removal, and nutrient addition (mow, roll, and nutrient addition); (2) a degraded (−N) riparian zone with continued compaction and vegetation removal, but without nutrient addition (mow and roll only); (3) a restored riparian area (no grazing, cessation of manure and N fertilizer application and re-vegetation with natural regrowth for 2 years); and (4) a reference riparian zone (no riparian zone degradation has occurred within the

last 10 years). Treatments 1, 2, and 3 were randomly assigned to 3 m × 9 m plots. There were no undisturbed riparian zones available on the treated side of the stream; hence, four reference plots (3 m × 9 m) were located in a riparian area on the other side of the stream.

We used the "mow and roll" treatment to separate physical (i.e., soil compaction + vegetation removal) versus chemical (i.e., nutrient addition) impacts of cattle grazing. Compaction was simulated by rolling plots receiving treatments 1 and 2 with a 200 kg roller 4 times per month. Vegetation removal was simulated by mowing plots 4 times per month during the growing season (March through November) with a lawn mower equipped with a grass catcher (clippings were removed from the study area). Nutrient addition from livestock grazing was simulated by adding sterilized cow manure at a rate of 132 kg N ha^{-1} per year and urea at a rate of 266 kg N ha^{-1} per year. These rates of N addition approximate expected inputs from a mid-size cattle operation.

Soil samples were collected quarterly starting from December 2001 (winter) with subsequent sampling in April (spring), July (summer) and October (fall) 2002. Hence, soil and microbial responses are representative of those occurring after 1.5–2 years of continuous treatment. Soil samples were taken to a depth of 10 cm using an auger in four treatments of four replicates. One composite sample in each treatment consists of 10 sampling points. After removing roots, fresh soil was passed through a 4 mm sieve and stored in a cold room. About 24 h before each microbial activity measurement, soil samples were removed from a cold room to allow microbes to be reactive at room temperature. Subsamples for chemical analyses were air-dried and ground to pass a 2 mm sieve.

Microbial biomass C was determined in samples of all periods. However, samples only from spring and summer were measured for nitrifiers and denitrifiers because of the excessive labor required for the most probable number (MPN) method. We also anticipated that nitrifier and denitrifier populations would be greatest during the warm and wet months, and hence prioritized our analyses for these spring and summer time periods. Nitrate concentration was measured for spring samples. Both spring and fall samples were analyzed for total N and C.

2.2. Laboratory analysis

2.2.1. Microbial biomass

Microbial biomass was estimated using $CHCl_3$ fumigation-extraction (Vance et al., 1987). After fumigation and extraction, potassium sulphate extractable C was determined using a Carlo–Erba NA 1500 model C/N analyzer.

2.2.2. Denitrifiers

Denitrifier densities were assessed using the method developed by Focht and Joseph (1973) and modified by Tiedje (1982). Screw top tubes were filled with 10 ml nitrate broth solution (prepared by mixing 8.0 g of nutrient broth and 0.5 g of potassium nitrate per liter water). Samples were dispersed by shaking for 10 min 5 g of fresh soil in 45 ml water plus 1 drop of Tween 80. After inoculation of sterilized tubes with 0.1 ml of appropriate dilutions, caps were tightened to exclude diffusion of atmospheric oxygen during the incubation. Five tubes per dilution were employed. After 14 days of incubation at 28 °C, 0.5 ml of medium was pipetted to test for NO_3^- and NO_2^- by adding 6 drops of diphenylamine reagent. A colorless response (no NO_3^- or NO_2^-) was considered as evidence of denitrification. Denitrifier number was then calculated using a MPN table (Cochran, 1950).

2.2.3. Nitrifiers

Ammonium sulphate was added in the medium of Martikainen (1985) for the determination of autotrophic ammonium oxidizers and sodium nitrite for autotrophic nitrite oxidizers. The MPN tubes were kept at 28 °C in an incubator for 8 weeks. The presence of nitrite or nitrate as the evidence of NH_4^+ oxidizers was checked by a drop test using the Griess–Ilosvay reagent (Schmidt and Belser, 1982). The ammonium oxidizer population was calculated using MPN table of Cochran (1950). Using the same approach, the number of NO_2^- oxidizers was estimated by the disappearance of NO_2^- as the evidence of NO_2^- oxidizers.

2.2.4. Soils

Total nitrogen and carbon in soil was determined using a Carlo-Erba NA 1500 model C/N analyzer. Nitrate in soil was determined colorimetrically in a

Lachat flow injector autoanalyser using 2 M KCl soil extracts.

2.3. Statistical analysis

Data were analyzed using analysis of variance (GLM procedure). Treatment differences were tested using Least Square Mean (LSMean procedure) pairwise comparisons. Nitrifier and denitrifier counts were transformed by $\log(X + 1)$ before statistical analysis. Statistical analyses were performed using SAS software (Littell et al., 1996). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

3. Results

3.1. Soil carbon and nitrogen

Comparisons of total soil N and C pools among treatments indicates very little impact on total pool sizes after 2 years of treatment. For example, although total soil N was significantly greater in the degraded + N plot versus the degraded − N and reference plots in the spring, these differences were not observed in the fall (Table 1). Total soil C was significantly greater in the restored plot compared to the reference plot in the fall, but no differences were observed in the spring (Table 1). The inconsistency of these response patterns, in combination with relatively small differences in pool sizes among treatments, indicates that the treatments have not yet had a significant affect on total soil N and C pools. In contrast, NO_3^- concentration in the degraded + N plots was nearly 10 times higher than that in the restored and reference plots, and four times higher than that in the degraded − N (Table 1). After two years of restoration, the restored plots had the same soil NO_3^- level as the reference plots.

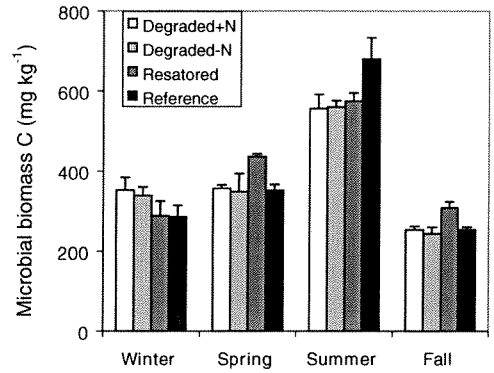

Fig. 1. Seasonal dynamics of microbial biomass in surface soil (0–10 cm) across all treatments. Bars represent S.E.

3.2. Microbial populations

Microbial biomass C varied significantly among sampling dates, but it did not differ among treatments (Fig. 1). In contrast, there was considerable variability in specific microbial populations that regulate many N cycling processes. For example, populations of denitrifiers, ammonium oxidizers, and nitrite oxidizers were consistently greater on the degraded + N treatment than all other treatments. With the exception

Table 1
Carbon and nitrogen (mean ± S.E.) of soils sampled in spring and fall

Treatments	Total N ($g\,kg^{-1}$)	Total C ($g\,kg^{-1}$)	NO_3^- ($\mu g\,g^{-1}$)
Spring			
Degraded + N	3.1 ± 0.2 a	31.0 ± 1.3 a	8.5 ± 2.4 a
Degraded − N	2.6 ± 0.2 bc	31.4 ± 1.3 a	2.4 ± 0.9 b
Restored	2.9 ± 0.2 ab	33.7 ± 1.9 a	0.8 ± 0.3 b
Reference	2.5 ± 0.1 c	32.1 ± 1.5 a	0.8 ± 0.4 b
Fall			
Degraded + N	2.7 ± 0.1 a	31.8 ± 1.0 ab	ND
Degraded − N	2.3 ± 0.2 a	27.6 ± 1.8 ab	ND
Restored	2.7 ± 0.2 a	32.0 ± 2.4 a	ND
Reference	2.2 ± 0.1 a	27.6 ± 1.2 b	ND

Numbers carrying the same letters in a column are not significantly differently [$P < 0.05$] based on the LSMean pairwise comparison. ND: not determined.

Table 2
Population [log(X + 1)] of denitrifiers, and nitrifiers (ammonium oxidizers and nitrite oxidizers) in surface soil (0–10 cm)

Treatments	Spring			Summer		
	Denitrifiers	Ammonium Oxidizers	Nitrite Oxidizers	Denitrifiers	Ammonium oxidizers	Nitrite oxidizers
Degraded + N	4.43 a	4.20 a	5.18 a	4.84 b	4.39 c	5.12 b
Degraded − N	3.86 ab	3.47 b	4.81 a	4.62 b	2.89 a	4.54 a
Restored	4.32 a	3.22 b	5.15 a	4.56 ba	2.96 a	4.68 a
Reference	3.60 b	3.46 b	5.04 a	3.66 a	3.60 b	4.57 a

X stands for number in a gram soil. Numbers carrying the same letters in a column are not significantly different at $P = 0.05$ based on the LSMean pairwise comparison.

of the spring sample period for nitrite oxidizers, the degraded + N treatment had significantly greater microbial populations than the reference plots (Table 2). More specifically, in the summer, the degraded + N plots had significantly greater populations of nitrifiers (i.e., ammonium oxidizers and nitrate oxidizers) than all other treatments (Table 2). Population sizes of nitrite oxidizers on the restored plots were not significantly different from the reference plots during either sample period; however, restored plots had significantly greater populations of denitrifiers in the spring and significantly lower populations of ammonium oxidizers in the summer (Table 2).

4. Discussion

Increased vegetation growth and productivity following restoration typically results in larger and more stable organic matter inputs from root and leaf litter. Despite favorable organic matter inputs during the 2 years after restoration treatments, microbial biomass levels in the restored plots were not significantly different from those in the degraded + N. Others have found a strong connection between increased organic matter and soil microbial activity and biomass. For example, Wardle et al. (2001) stated that practices which result in a greater addition of basal resource are likely to stimulate soil microflora. Haycock and Pinay (1993) found that organic C input promotes the formation of microbial biomass, leading to great efficiency of N conservation in vegetated sites. The results of our study showed that total soil microbial biomass did not respond to restoration after 2 years. Instead, several years of restoration may be required to replenish soil organic matter, with subsequent increases in microbial biomass in response to more stable OM inputs.

The significant seasonal differences in soil microbial biomass observed in this study were most likely due to variations in soil temperature. For example, mean air temperature in the region varies from 2.6 °C in January, 12.5 °C in April, 21.6 °C in July to 12.9 °C in October (L.W. Swift Jr., unpublished data) and similar surface soil temperature variation would be expected (Vose and Swank, 1991). The highest microbial biomass in summer corresponded to the highest temperature of a year (July). Higher precipitation in winter months (L.W. Swift Jr., unpublished data) probably reduced the negative impact of low temperature and maintained a microbial biomass level comparable to spring and fall. Variations in root biomass and rhizosphere products (Franzluebbers et al., 1994) could be other causes of seasonal change in microbial biomass besides air temperature.

Differences in soil microbial populations between degraded + N and restored plots is indicative of a restoration response. A lower population of nitrifiers in the degraded − N, restored, and reference plots is most likely attributed to reduced N inputs as a result of cattle exclusion. At Rothamsted Experimental Station, England, Willison et al. (1997) reported that unfertilized arable soils had lower numbers of bacteria, including ammonium oxidizers, than fertilized ones. Aarnio and Martikainen (1992) observed that urea application enhanced the build-up of nitrifiers in a forest ecosystem in southern Finland. Because nitrifiers oxidize NH_4^+ to form NO_3^-, a reduction in nitrifier populations after riparian zone restoration may have contributed to the observed reduction in soil NO_3^-. This reduction is especially important for maintaining low surface and groundwater N levels because of the

mobility and toxicity of NO_3^-. In addition, since NO is a by-product of the nitrification process (Anderson and Levine, 1986), reduced nitrification in restored riparian areas may reduce atmospheric NO emissions.

In conclusion, restoration of degraded riparian zones led to a reduction in nitrifier numbers which has important implications for water and air quality. Further studies are needed to look into the diversity of denitrifiers and nitrifiers as difference in species compositions could potentially influence the N transformation (Cavigelli and Robertson, 2001) in addition to population numbers.

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