ROOT BIOMASS AND DENITRIFICATION POTENTIAL IN DEGRADED AND RESTORED URBAN RIPARIAN ZONES

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INTRODUCTION

Nitrate is the most common stream pollutant in the U.S. (USEPA, 1990). It enters groundwater through runoff from fertilizer use, leaching from septic tanks and sewage, and the erosion of natural deposits, and is especially problematic downstream of urban and agricultural areas, hotspots for nitrate contribution. High levels of this ion can cause stomach cancer and birth defects in humans, the most common being methemoglobinemia, or "blue baby syndrome" (Camargo and Alonso, 2006; Follett and Hatfield, 2001). In addition to these health effects, high levels of nitrate cause eutrophication in the bodies of water that are fed by polluted streams. Known as cultural or anthropogenic eutrophication, this process causes lakes, streams, and bays to age at faster rates than normal. The increased amount of nutrients causes eutrophication and hypoxic conditions which stimulate the growth of algal blooms interfering with the health, diversity, and fitness of native plants and animals, aquatic and terrestrial (National Science and Technology Council Committee, 2003).

Denitrification is an important process in urban coastal regions because it reduces nitrate into nitrogen gas, thereby removing it from the stream and riparian areas. This process requires certain soil conditions: a high water table, alternating periods of aerobic and anaerobic conditions, populations of denitrifying bacteria and sufficient amounts of organic carbon (Paul and Clark, 1996). Most of the microbes that are integral to denitrification are heterotrophic which means that they use organic compounds as energy sources. Therefore we expect high rates of denitrification in wetland soils which tend to be anoxic and carbon rich (Groffman and Crawford, 2003).

In urban areas, the denitrification potential of streams and riparian zones is often decreased due to a suite of degradation effects collectively referred to as "urban stream syndrome" (Walsh, et al, 2005). Urban streams are often characterized by extreme incision and bank erosion due to storm water runoff from impervious surfaces in urban watersheds. This incision, combined with reductions in infiltration in the uplands, leads to lower water tables (hydrologic drought) and drier (more aerobic) soils in urban riparian zones, with less denitrification (Groffman et al. 2003).

Efforts to reverse urban stream syndrome through geomorphic restoration are active in many areas (Bernhardt et al., 2005). These efforts include stabilization of banks, reversing incision by filling or raising the channel and reconstructing pool and riffle features. These efforts have the potential to raise the water table in the riparian zone and restore denitrification. While much research has explored denitrification potential in the riparian zones surrounding urban streams and forested natural streams (Groffman, et al. 2002, Groffman, et al. 2003, Groffman and Crawford, 2003), no measurements have been made in the restored riparian zones.

In addition to anoxic soils created by higher water table levels, denitrification also requires available organic carbon. There has been concern regarding organic carbon sources for the denitrifiers in restored riparian zones. The materials used in the restoration and reconstruction process are low in carbon and therefore would not be good carbon sources for denitrification. In riparian zones, roots are a potentially important source of carbon and this paper deals with the question of how quickly carbon builds up in riparian zones by measuring the amount of root biomass present at the urban, restored, and forested stream sites. This is an important question due to the increased concern over eutrophication in the Chesapeake Bay and interest in determining if current restoration efforts have the potential to restore urban stream function.

By measuring denitrification potential and root biomass in the riparian zones of forested reference, urban degraded and restored streams, I addressed the following questions:

1. Does the denitrification potential in the riparian zones around restored streams begin to mimic that of the forested natural zones or does it still look more like the amounts found in degraded urban zones?
2. How does the amount of root biomass differ between urban, restored, and forested sites, if at all?
3. Does stream restoration help restore the natural microbial processes and root biomass in riparian areas?
4. If so, how long does it take for this process to mimic natural riparian areas?

I hypothesized that there is a gradient of recovery between degraded urban and forested natural riparian zones and that denitrification potential and root biomass in the restored areas will increase with time since restoration, but will still be lower than in natural reference areas.

METHODS AND MATERIALS

Sampling Sites

Six riparian sites were sampled in Baltimore City and Baltimore County, MD. All study sites are currently being researched as part of the National Science Foundation funded urban long-term ecological research project, the Baltimore Ecosystem Study (BES, http:beslter.org) and were located in or near the Gwynns Falls watershed (76°30', 39°15' and approximately 17,150 hectares). They included two forested, two urban, and two restored streams and their riparian zones (see Table 1). Pond Branch (POBR) and Baisman Run (BARN) are predominately forested sites located in Baltimore County in the Baisman Run watershed. BARN does have a residential area with septic tanks which contributes to higher nitrate levels. Glyndon (GFGL) and Gwynnbrook (GFGL) are characteristic suburban sites with high levels of nitrate. Both are located within Gwynns Falls itself. Finally, Mine Bank Run (MBRN) and Spring Branch (SPBR) are restored sites are also located in Baltimore County near Timonium, MD. The EPA's Spring Branch Stream Restoration project began in 1994 and was completed in 1997 (EPA, 2002). MBRN was completed within the last five years.

Half of the sites had long-term groundwater monitoring wells (POBR, GFGL, and GFBG) already established by BES (Groffman et al., 2002) and all sites had extensive background data on stream chemistry.

METHODS

Four 1-meter long soil samples were taken from each site using a JMC, Inc. E environmentalist's Subsoil Probe (ESP) to retrieve intact cores. At the streams with long-term groundwater monitoring wells, each core was taken within three meters of the well. At the remaining streams, two sites were established approximately 50 meters from one another. Two cores were taken at each site on opposite sides of the stream (if possible), between 3 to 5 meters from the water edge (see Figure 1 for general layout). At BARN, the sites were located between the long-term USGS stream gage and the confluence with Pond Branch. At MBRN, the sites were located near existing sites of push/pull measurements done by BES, and at SPBR, the sites were established along the restored reach of the stream. In addition to the soil cores, two pictures were taken at each sampling site, one upstream and one downstream. If bedrock was encountered at any site that made it impossible to retrieve an entire 1-meter length of oil, a partial core was taken and depths were recorded. In the case of compaction (especially in very wet soils), the 1-meter depth was achieved and the soil sample was further assessed in the lab.

Each core was labeled and refrigerated at 4°C until transport from Baltimore to Millbrook, NY and then stored in a cold room (4°C) for approximately 2 weeks until lab analysis was done. Once in the lab, the cores were cut into 4 sections (0-10 cm, 10-30 cm, 30-70 cm, and 70-100 cm) based on length, possible compaction, texture, and color and each section was weighed, divided into two samples, and stored in a ziptop plastic bag. Roots and rocks larger than 2 mm were removed prior to weighing. The samples were then kept refrigerated (4°C) until all tests were completed.

Soil moisture content was determined drying 5 g of soil from each sample at 105°C for 24 hours (McInnes, et al., 1994). These samples were then used for determination of organic matter content by loss on ignition at 450°C for 4 hours (Nelson and Sommers, 1996).

Denitrification enzyme activity (DEA) was measured using the short-term anaerobic assay developed by Smith and Tiedje (1979) as described by Groffman et al. (1999). Two replicate samples were amended with NO_3^-, dextrose, chloramphenicol, and acetylene and incubated under anaerobic conditions for 90 minutes, with gas samples taken at 30 and 90 minutes, stored in evacuated glass vials, and then run on an electron capture gas chromatograph to determine N_2O levels.

Roots in the sample were removed by hand (a 15 minute time limit for root picking was established), rinsed twice (once by dipping into deionized water and once by shaking in deionized water), dried at 60°C for 24 hours (Rotkin-Ellman, et al, 2004), and weighed.

Statistical Analysis

DEA, root biomass, and LOI were compared with a two-way analysis of variance with land use setting (natural, urban, and restored) and soil depth as main effects. Relationships between variables were explored with linear (Pearson) and non-parametric (Spearman) correlation. The SAS statistical program was used for these analyses (SAS Institute, 1988).

RESULTS

Mean denitrification potential across all soil depths ranged from 9.3 ng N g^{-1} h^{-1} in a forested/natural (POBR) site to 226 ng N g^{-1} h^{-1} in a restored site (SPBR). Root biomass ranged from 0.81 g kg^{-1} at SPBR to 4.1 g kg^{-1} at BARN (forested/natural). The 70 – 100 cm depth at BARN, a forested/natural site, had significantly higher amounts of root biomass than any of the other sites due to the presence of one coarse root in one of the samples from this site. This sample was removed from any analyses involving root biomass.
Soil moisture content was lowest at MBRN (new restored, 163 g kg^{-1}) and highest at POBR (272 g kg^{-1}). Soil organic matter content ranged from a low of 16 g kg^{-1} at SPBR to a high of 33 g kg^{-1} at GFGB (urban/degraded).

There were strong positive relationships between root biomass and organic matter content (Figure 2, $r^2 = 0.60$, $p < 0.0001$), denitrification potential and organic matter content (Figure 3, $r^2 = 0.73$, $p < 0.0001$) and denitrification potential and root biomass (Figure 4, $r^2 = 0.41$, $p < 0.0001$) (Table 3.)

Denitrification potential varied with both depth and stream type. Potential was significantly ($p<0.0001$) higher in the top 10 cm of soil across all sites. Although no significant difference occurred between the sites, the restored streams did show a trend of higher denitrification potential. Within the restored streams, SPBR (restored 10 years ago) showed a trend of higher potential than MBRN (restored 5 years ago) (Figure 5).

Soil organic matter decreased with soil depth, but not nearly as dramatically as denitrification potential (Figure 7). With the exception of the top layer of soil, the urban/degraded sites had the highest, and the restored sites had the lowest organic matter contents. Significant differences occurred within the lower layers, however. The 10-30 cm layer showed the restored sites to be significantly ($p<0.05$) lower than the other two sites. In the 30-70 cm layer, the urban sites were significantly ($p<0.01$) higher than the restored sites. And, finally, the urban sites were significantly ($p<0.01$) higher than the other two sites.

As was the other variables, root biomass decreased significantly ($p<0.0001$) with depth (Figure 8). The forested reference sites had the highest, and the restored sites had the lowest root biomass, especially at depth (Figure 8).

DISCUSSION

The lack of significant differences in denitrification among sites and land use classes was likely due to the complex regulation of this process by oxygen, carbon and nitrate in nature. While the forested reference sites had the highest levels of soil moisture (low oxygen) and organic carbon, they had very low levels of nitrate and therefore low denitrification potential. While the urban degraded restored sites had low levels of organic carbon, as expected, the high levels of nitrate in these sites stimulated denitrification potential. As a result, there was no clear pattern in denitrification among the land use classes in this study.

My data shows that there is no significant difference in denitrification potential between the three land use types presented in this paper; however, the data does indicate a general trend. The restored type shows a higher potential for denitrification than the urban or forested sites. This is most likely due to the fact that the urban and restored sites are exposed to more nitrate than the forested zones. Being that nitrate is one of the three requirements for denitrification to occur, this could be a limiting factor for denitrification in the forested sites. Kaushal, et al (2004) found that more nitrate was found in unrestored sites than in restored sites and consequently higher rates of denitrification were found in the restored sites and lower in the unrestored.

Although no significant difference in denitrification potential was found between sites or land use classes, there was significant variation with depth. The rate of N_2O production resulting from the denitrification enzyme assay was considerably higher in the top 10 cm of soil than in any of the other layers. It was also quite common to find little to no N_2Oproduced in the very lowest layer, 70-100 cm below the soil surface. This indicates that the top 10 cm of soil is the more important layer to consider during restoration processes. When looking at the BES well data from October 2005 to August 2006 (Appendix A), the urban/degraded sites (GFGB and GFGL) consistently had lower water tables than the forested/natural site (POBR). Because the water is lower it cannot contribute to creating the anaerobic conditions needed for denitrification in the upper layers of soil. Although this may seem counterintuitive and that denitrification should occur at higher levels in lower soil layers, the top layer contains more root biomass and organic carbon, implying that organic matter could be a limiting factor in the lower soil layers.

My data also shows that root biomass is highly correlated with organic carbon content, meaning that root biomass is a significant source for carbon in the soil. Similarly, there was a strong, positive relationship between organic carbon content and denitrification potential. This relationship is consistent with many other studies (Groffman and Crawford, 2003) and was expected. In theory because these two relationships occurred so strongly, it would seem that root biomass should be just as strongly correlated meaning that higher amounts of root biomass would occur in the same places where higher rates of denitrification potential occur. In truth, there is a positive relationship between these two factors, but the correlation is not as strong ($r^2=0.4116$) as with soil organic carbon. These three relationships: roots vs carbon content, carbon content vs denitrification potential, and roots vs denitrification potential, are part of a three step process. Higher amounts of roots lead to more organic carbon content which leads to a higher denitrification potential. The relationship between roots and denitrification potential is indirect which is why the relationship is weaker than the others.

According to Pouyat, et al (2002), carbon sources in urban landscapes are constantly in flux due to the flashiness of urban streams. This flashiness, usually associated with high storm flows, causes bank incision and erosion, which in turn, reduces the amount of carbon in the soil. The restoration process stabilizes these banks and, therefore, stabilizes the carbon sources in the riparian zones of restored streams. When looking at the denitrification potential rates by land use, it seems that more denitrification can occur in the restored sites indicating that restoration practices may have benefited the microbial processes in the restored sites studied in this project. This is probably due to raised water tables (and therefore, anaerobic soils) and a more protected carbon source.

Regardless of this optimistic result, compared to the other land use types, the restored sites had less root biomass and soil organic carbon. This is extremely important to note because more root biomass leads to more denitrification. The restored and urban sites are exposed to more nitrate which would increase the denitrification potential of those riparian zones, but because there is less organic carbon and less root biomass at the restored sites, it seems that root biomass could be limiting factors in actual denitrification. Restoration methods should concentrate on encouraging root growth at all soil depths in order to increase the amount of denitrification occurring.

There is a strong indication that time is necessary for the restored riparian zones to build this foundation of organic matter. SPBR was restored roughly 10 years ago and MBRN was done less than 5 years ago. SPBR yielded a denitrification potential that is three times that of MBRN. Using this data, it seems that the longer time a restored site has to "settle", the more benefit to microbial processes exists. Ultimately, the restored sites need more time to develop a good foundation of organic carbon in the soil in order for these streams to be fully effective in removing nitrate.

Due to the importance of riparian zones as a sink for nitrate, it is imperative that restoration activities continue. Over the past 15 years, the U.S. government has spent more than $7.5 billion on riparian restoration but has only monitored roughly 10% of the projects it has funded (Voshell and Braccia). Because of the economical and ecological value of wetland restoration, further research of restored riparian zones and proper restoration practices are crucial. In order to further the effectiveness of riparian restoration, managers must ensure a suitable denitrification environment. Restoration must raise the water table (creating anaerobic conditions), stabilize the stream banks (reducing potential for soil organic matter flux), and make certain that vegetation is established and sustainable.

CONCLUSION

- Complex control of denitrification makes it difficult to compare reference, degraded and restored sites.
- Strong declines in denitrification, organic matter and roots with depth reinforce the idea that water table is key controller of riparian denitrification and should be a target for restoration.
- Restored sites have low root biomass and organic matter content, even 10 years following restoration, although they show a positive trajectory.

In conclusion, the restoration process does seem to help increase the denitrification potential of degraded, urban streams. There are both physical and microbial benefits attributed to restoration. Water tables have risen (Appendix A) and denitrification potential has increased. Both of these factors can help reverse the effects of urban stream syndrome which, in turn, shows the potential to reverse cultural eutrophication.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Foundation (NSF) Research Experiences for Undergraduates (REU) program (2006-on Grant No. DBI-0552871). This is a contribution to the program of the Cary Institute of Ecosystem Studies.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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APPENDIX

TABLE 1. Stream sites used in Baltimore County and Baltimore City.

Site	Type	Previous water table data
Pond Branch (POBR)	Forested	√
Baisman Run (BARN)	Forested	
Glyndon (GFGL)	Urban	√
Gwynnbrook (GFGB)	Urban	√
Mine Bank (MBRN)	Restored (new)	
Spring Branch (SPBR)	Restored (old)	

TABLE 2. Mean denitrification potential, root biomass, soil moisture content and organic matter content in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area.

	Forested/Natural		Urban/Degraded		Restored	
Variable	POBR	BARN	GFGL	GFGB	MBRN (new)	SPBR (old)
Denitrification potential, ng N g^{-1} hr^{-1}	9.28 (7.32)	90.24 (76.02)	128.36 (117.45)	60.76 (55.18)	71.32 (69.34)	225.72 (185.27)
Root biomass, g kg^{-1}	2.06 (1.33)	4.13 (1.11)	0.78 (0.46)	2.58 (1.35)	1.50 (0.48)	0.81 (0.66)
Soil moisture content, g kg^{-1}	272 (65)	240 (23)	186 (14)	270 (13)	163 (15)	221 (24)
Soil organic matter, g kg^{-1}	28 (12)	22 (4)	24 (4)	33 (4)	28 (11)	16 (6)

TABLE 3. Statistical relationships between denitrification potential, soil organic matter, and root biomass for 6 riparian soil profiles in the Baltimore metropolitan area.

All at p<0.0001 (Spearman correlation)

	Denitrification Potential	Organic Matter	Root Biomass
Denitrification Potential	--	0.58	0.39
Organic Matter	0.58	--	0.52
Root Biomass	0.39	0.52	--

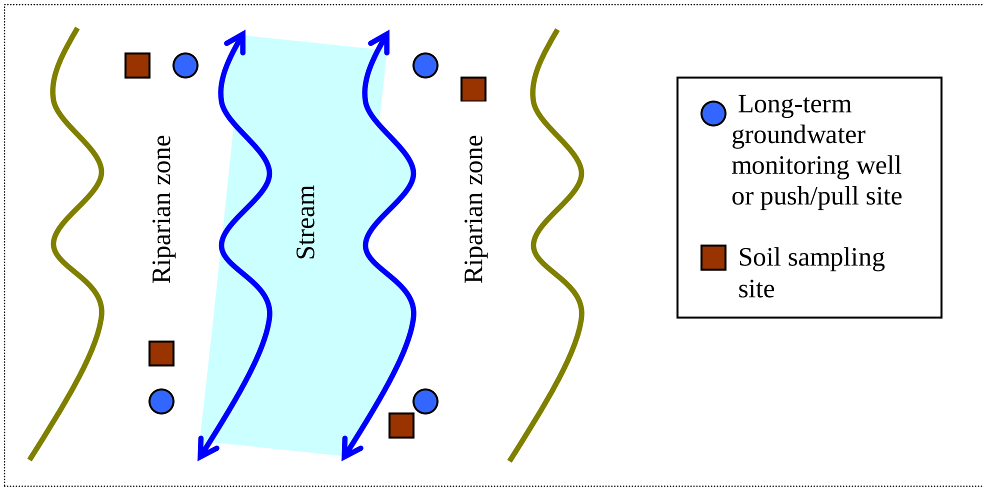

FIGURE 1. General location of soil extraction sites relative to current wells. The set-up for sites that do not have wells was similar. Sampling sites were established 3-5 m from the stream edge. Two were located across from one another and the other two were located at least 50 m downstream from these sites, also directly across the stream from one another.

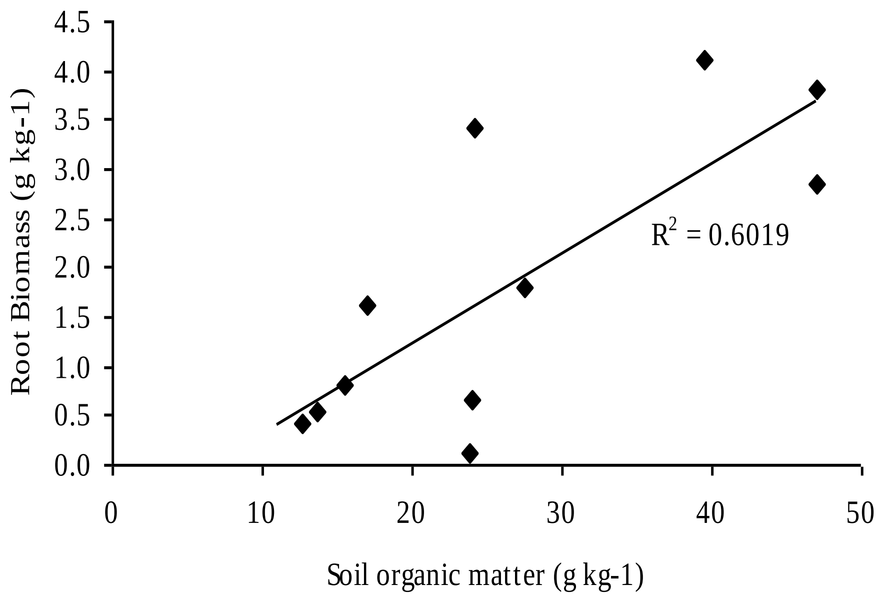

FIGURE 2. Root biomass versus soil organic matter in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area.

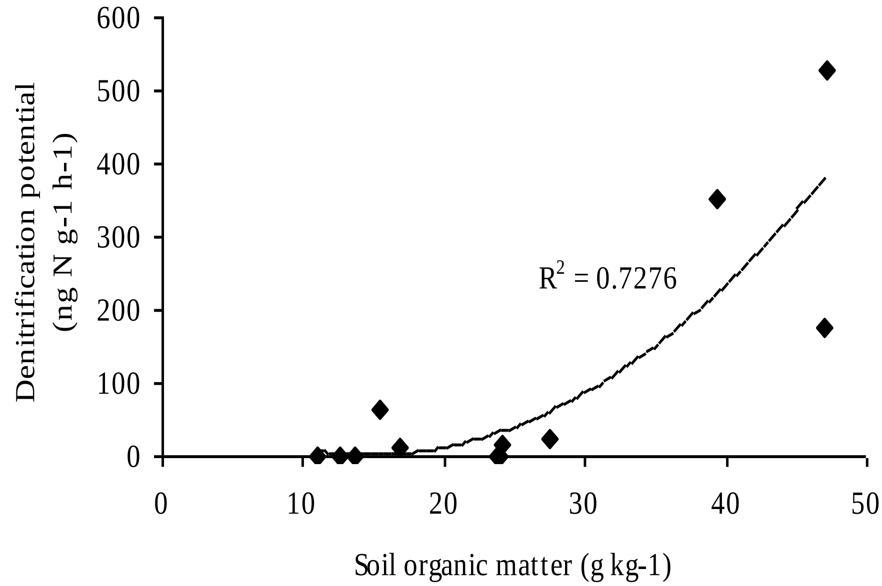

FIGURE 3. Denitrification potential versus soil organic matter in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area.

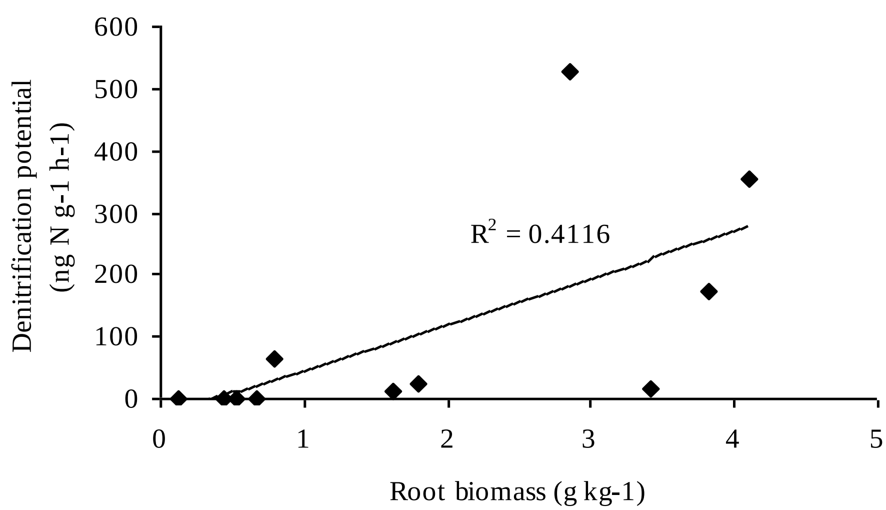

FIGURE 4. Denitrification potential versus root biomass in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area.

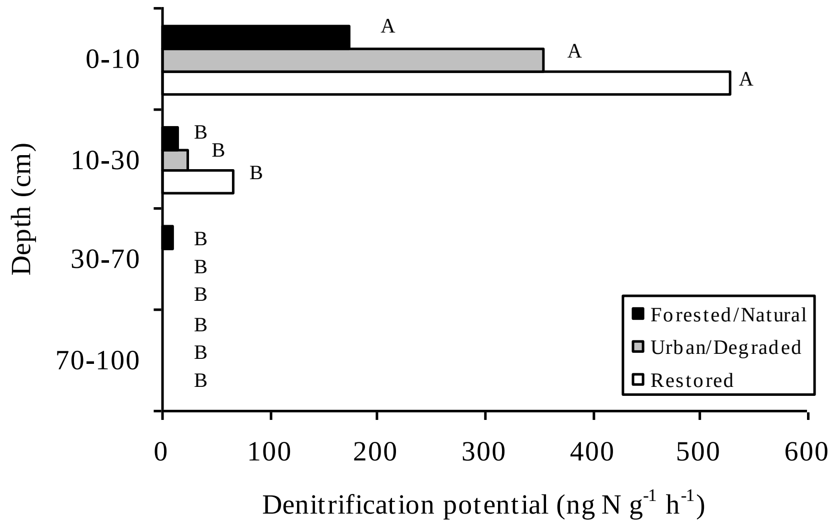

FIGURE 5. Denitrification potential at four depths in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area. There is no significant difference between stream sites; however, the top 10 cm of the soil does have significantly higher denitrification potential than in the other layers.

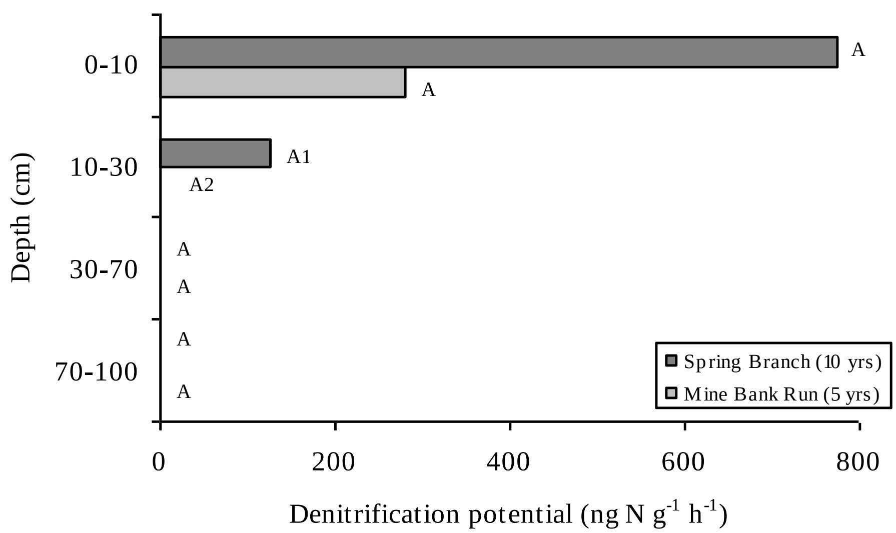

FIGURE 6. Denitrification potential at four depths in 10 year and 5 year old restored riparian zone soil profiles in the Baltimore metropolitan area. No significant difference occurs between the sites except for the 10-30 cm depth.

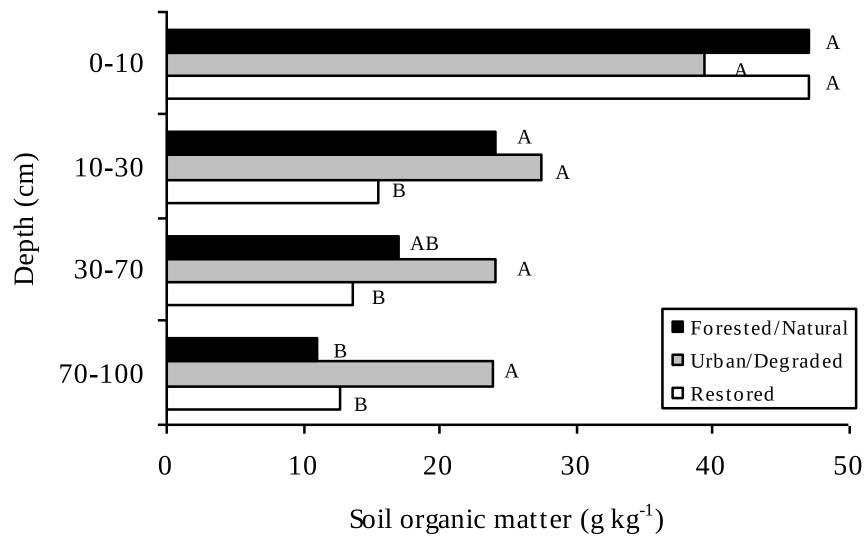

FIGURE 7. Soil organic matter at four depths in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area. The 0-10 cm layer had significantly higher SOM than the lower soil layers. Significant differences occurred within each layer as well.

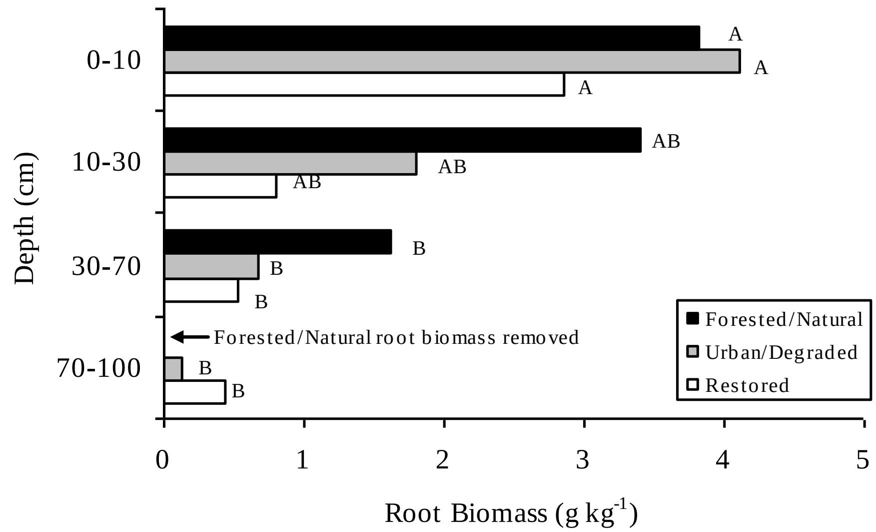

FIGURE 8. Root biomass at four depths in forested reference, urban degraded and restored riparian zone soil profiles in the Baltimore metropolitan area. Data from the 70 – 100 cm depth for the forested reference sites are not shown because the values were so much higher than other values on the Figure (*BARN contained 6.74 g kg^{-1} of root biomass compared to a range of 0.015 - 0.852 g kg^{-1} in the other sites*).

BES RIPARIAN WELL DATA

Measurements for the June 2006 data were taken by this author when soil collection was done at each site. Cahill is an urban site not included in this study.

Oct-05

Site	Average of Depth (mm): lip to H20
Cahill	893.75
Glyndon	1023.50
Gwynnbrook Pond	1007.00
Branch	641.00

Dec-05

Site	Average of Depth (mm): lip to H20
Cahill	738.33
Glyndon	621.25
Gwynnbrook Pond	
Branch	629.50

Jan-06

Site	Average of Depth (mm): lip to H20
Cahill	888.00
Glyndon	572.50
Gwynnbrook Pond	725.00
Branch	671.33

Feb-06

Site	Average of Depth (mm): lip to H20
Cahill	826.25
Glyndon	480.00
Gwynnbrook Pond	855.00
Branch	655.00

Mar-06

Site	Average of Depth (mm): lip to H20
Cahill	
Glyndon	1035.00
Gwynnbrook Pond	1075.00
Branch	652.50

Apr-06

Site	Average of Depth (mm): lip to H20
Cahill	1010.00
Glyndon	967.50
Gwynnbrook Pond	1069.50
Branch	643.75

May-06

Site	Average of Depth (mm): lip to H20
Cahill	907.50
Glyndon	1178.75
Gwynnbrook Pond	1148.75
Branch	623.75

Jun-06

Site	Average of Depth (mm): lip to H20
Cahill	1081.67
Glyndon	1177.50
Gwynnbrook Pond	1183.75
Branch	651.25

Aug-06

Site	Average of Depth (mm): lip to H20
Cahill	1211.67
Glyndon	1290.00
Gwynnbrook Pond	1223.33
Branch	835.00