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Denitrification and Nitrous Oxide Emissions from Riparian Forests Soils Exposed to Prolonged Nitrogen Runoff

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ABSTRACT

Compared to upland forests, riparian forest soils have greater potential to remove nitrate (NO_3) from agricultural run-off through denitrification. It is unclear, however, whether prolonged exposure of riparian soils to nitrogen (N) loading will affect the rate of denitrification and its end products. This research assesses the rate of denitrification and nitrous oxide (N_2O) emissions from riparian forest soils exposed to prolonged nutrient run-off from plant nurseries and compares these to similar forest soils not exposed to nutrient run-off. Nursery run-off also contains high levels of phosphate (PO_4). Since there are conflicting reports on the impact of PO_4 on the activity of denitrifying microbes, the impact of PO_4 on such activity was also investigated. Bulk and intact soil cores were collected from N-exposed and non-exposed forests to determine denitrification and N_2O emission rates, whereas denitrification potential was determined using soil slurries. Compared to the non-amended treatment, denitrification rate increased 2.7- and 3.4-fold when soil cores collected from both N-exposed and non-exposed sites were amended with 30 and 60 μg NO_3-N g^{-1} soil, respectively. Net N_2O emissions were 1.5 and 1.7 times higher from the N-exposed sites compared to the non-exposed sites at 30 and 60 μg NO_3-N g^{-1} soil amendment rates, respectively. Similarly, denitrification potential increased 17 times in response to addition of 15 μg NO_3-N g^{-1} in soil slurries. The addition of PO_4 (5 μg PO_4–P g^{-1}) to soil slurries and intact cores did not affect denitrification rates. These observations suggest that prolonged N loading did not affect the denitrification potential of the riparian forest soils; however, it did result in higher N_2O emissions compared to emission rates from non-exposed forests.

Introduction

Extensive agricultural activities accompanied by the use of nitrogen (N) fertilizer have resulted in higher concentration of nitrate (NO_3) in surface waters in the U.S. (Vitousek et al. 1997; Mitsch et al. 2001; Turner and Rabalais 2003). Among agricultural activities, ornamental plant nurseries use more fertilizer than is used to cultivate row crops in the U.S. (Colangelo and Brand 2001). Both NO_3 and ammonium (NH_4) are highly prone to leaching from soilless growing media in plant nurseries under intensive irrigation regimes (Harris et al. 1997). Loss of mineral N from nurseries occurs intermittently after irrigation or heavy rainfall (Harris et al. 1997; Colangelo and Brand 2001). The N-laden runoff often flows across the nursery to finally reach bodies of water, contributing to the increasing reactive N load of surface and groundwater resources of the country (Galloway et al. 2004). Higher NO_3 concentration in the rivers of the U.S. is a major cause of eutrophication in coastal waters (Turner and Rabalais 1994; Day et al. 2003).

Denitrification, or reduction of NO_3 to N_2O and N_2 gases, is one of the major microbial processes in riparian forest soils (Hunter and Faulkner 2001). It occurs under anaerobic conditions in which organic carbon is used as an energy source and NO_3 as the terminal electron acceptor by heterotrophic soil bacteria (Tiedje, 1982). Riparian forest soils have greater potential to denitrify NO_3 than surrounding agricultural lands (Lindau et al. 1994; Delaune et al. 1996). Use and restoration of riparian forests as a nutrient management tool for removing NO_3 from agricultural and urban runoff is highly recommended to protect and improve water quality in the U.S. (Mitsch et al. 2001; Day et al. 2003).

Although riparian soils denitrify NO_3 at higher rates due to saturated soil conditions and greater quantities of microbially available carbon, NO_3 content under normal conditions can be limiting (Lowrance et al. 1995). Thus, an external source of NO_3 is needed to maintain high denitrification rates (Ullah et al. 2005) in these soils. Such loading of runoff NO_3 into N-limited riparian forests markedly enhances denitrification rates (DeLaune et al. 1996), but it is not clear whether chronic exposure to higher NO_3 runoff has a positive or negative impact on denitrfier activity in soils (Smolander et al. 1994; Hanson et al. 1994a; Ettema et al. 1999). Bowden et al. (2004), Compton et al. (2004), and Wallenstein et al. (2006), observed significantly reduced microbial biomass carbon and activity in N-enriched temperate forest soils compared to control plots. This suggests that prolonged exposure of natural ecosystems to N can influence important microbial functions in soil. Discerning the effects of chronic NO_3 loading on denitrifier activity in riparian forest soils is crucial to quantify the potential of riparian buffers to remove NO_3. As denitrification is extremely variable both temporally and spatially (Groffman et al. 1991), it would be useful to investigate the effects of episodic higher NO_3 loading, as occurs from plant nursery runoff after irrigation or rainfall, on denitrification rates of riparian forest soils (Groffman, et al. 1991). Such information would help to develop nutrient management strategies for agricultural runoff.

The relative amounts of N_2O and N_2 gases produced during denitrification in soils (Skiba et al. 1998) depends mainly on soil moisture, available carbon substrate, and NO_3 concentration (Breitenbeck et al. 1980; Linn and Doran 1984; Skiba et al. 1998). Higher soil moisture and available organic carbon substrate promote complete reduction of low to moderate levels of NO_3 to N_2 gas, thus reducing the net amount of N_2O produced

(Linn and Doran 1984; Ullah et al. 2005). Higher levels of soil NO_3, however, result in higher net $N_2O:N_2$ gas emission ratios, since reduction of NO_3 compared to N_2O is more energy efficient and is favored by denitrifiers (Breitenbeck et al. 1980; Ullah et al. 2005). Thus, denitrification in riparian forest soils exposed to prolonged NO_3 runoff may result in higher net N_2O emissions (Fenn et al. 1998). N_2O is a 'greenhouse gas' that can induce 310 times more global warming than CO_2 on a mole-per-mole basis and thus can upset the credits gained from atmospheric CO_2 sequestration in these ecosystems (IPCC 1996; Yu et al. 2004). Moreover, N_2O is also a major contributor in depleting stratospheric ozone (IPCC 1996). Current efforts to sequester atmospheric CO_2 into restored riparian wetland soils may be jeopardized by increased N_2O emissions from these same ecosystems. There is an acute paucity of data on N_2O emissions from riparian forests in the northeastern U.S. (Groffman et al. 2000a), particularly from those exposed to prolonged NO_3 loading. Lack of data on the dynamics of N_2O emissions from riparian forests has hampered efforts to accurately measure and model N_2O emission factors from riparian zones for nitrogen cycling budgeting on a landscape scale (Groffman et al. 2000a).

In addition to NO_3, agricultural runoff also carries phosphorus (P), which, as a pollutant, can affect water quality and other factors in aquatic ecosystems (Silvan et al. 2003; Sudareshwar et al. 2003). Since P is an integral part of the microbial biomass in soils, prolonged P loading into riparian forest soils may affect the activity of soil microbes, including denitrifiers (Silvan et al. 2003; Meyer et al. 2005). There are conflicting reports on the effect of soil P level on the activity of denitrifiers. Sudareshwar et al. (2003) observed a decrease in denitrification rates when coastal wetland soils were amended with P compared to soils with limited P; alternatively, Federer and Klemedtsson (1988) and White et al. (2001) did not observe any effect of additional P on denitrifer activity in upland forest and Florida Everglade wetland soils, respectively. It would of interest to know if prolonged P loading of riparian forest soils impacts denitrifier activity.

In this study, we compared the effect of additional NO_3 on denitrification and net N_2O emission rates from riparian forest soils exposed to prolonged mineral N loading from plant nurseries. In addition, the impact of phosphate amendments on denitrification rates at selected sites was also evaluated.

Material and Methods

Study sites

Four riparian forest sites were identified in southern New Jersey in the upper Cohansey River watershed (located between 75° 5' to 75° 20' W longitude and 39° 22' to 39° 35' N latitude). Two of the sites, Loew forest (LF) and Centerton forest (CF), were exposed to nutrient runoff from surrounding plant nurseries for a period of 10 years. The other two sites, Natural forest (NF) and Harmoney forest (HF), are located within 0.5 and 3 miles of the LF site and did not receive runoff from surrounding nurseries or landscapes for this period. As such, these sites are considered as non-exposed in terms of chronic mineral N loading from the surrounding acreage. Atmospheric N deposition in New Jersey range from 3.6 to 7.8 kg N ha^{-1} y^{-1} (Dighton et al. 2004). This range of atmospheric N deposition in the region is considered elevated due to increased fossil fuel combustion and fertilizer production and use in the past 50 years (Fenn et al. 1998; Venterea et al. 2003). This may have deleterious impacts on soil N cycling in riparian forest soils in southern New Jersey, in addition to the nursery run-off N entering into some of the riparian buffers.

Runoff reaching the N-exposed sites arose mainly from frequent over-head sprinkler irrigation (at least twice-weekly from May to September) and rainfall from 150 acres of container grown and field nursery crops (LF) or 200 acres of container grown crops (CF). The runoff entered the LF site through a drainage PVC pipe and the CF site through a drainage ditch. Four replicate samples of runoff water were analyzed for NO_3 concentration at both locations in May and June, 2005 using the Flow Injection Analyzer at the Rutgers University Soil Analysis laboratory. The average NO_3 load of drainage entering the LF site was 15.0 and 8.2 mg L^{-1} while that entering the CF site was 3.0 and 12.5 mg NO_3 L^{-1}, which in some cases exceeded the EPA water quality standard of 10 mg L^{-1} (EPA 2004).

Due to lack of availability of analytical data on the extent and duration of run-off nitrate entering these sites, an indirect approach was adopted. Pools of N in soil and foliar litter were investigated for signs of prolonged nitrogen exposure and saturation. An increase in foliar nitrogen content, nitrification rates and NO_3 leaching from forests in response to chronic N loading are the established primary indicators of N saturation (Aber et al. 1989; Magill et al. 2000).

The soils in the four sites range in texture from silty clay loam to loamy sand. All supported mature forests, not used for commercial forestry, that were dominated by mature stands of hardwood tree species of white oak (*Quercus alba*), northern red oak (*Q. rubra*), red maple (*A. ruburum*), silver maple (*A. saccharinum*), willow oak (*Q. phellos*), pin oak (*Q. palustris*), and American holly (*Ilex opaca*). Other non-dominant tree species in these forests are green ash (*Fraxinus pennsylvanica*), white ash (*F. americana*), yellow popular (*Liriodendron tulipifera*), sweet gum (*Liquidamber styraciflua*), American elm (*Ulmus americana*), and bitternut hickory (*Carya cordiformis*). The LF site was infested with reeds *(Phragmites australis*), growing as a sub-canopy under the hardwood trees, that were concentrated along the nursery runoff flow path within the site. The CF site had relatively higher snag density and woody debris biomass than the other sites. Selected physico-chemical properties of the four sites are shown in Table 1. Consistently higher potential nitrification rates, % foliar N and soil mineral N, and lower C:N ratios in the N-exposed sites compared to the non-exposed sites shows that the LF and CF sites were exposed to prolonged mineral N loading (Table 1).

Soil sampling

Four replicate 1 m^2 sampling plots were randomly located at each site. Plots at the LF and CF sites were located in forest areas inundated by the nursery runoff sheet flow. To avoid edge effects on soil characteristics, the randomly placed plots were situated in a line at least 16 m down the boundary of the surrounding land uses and the forest. Unusual features such as hoof prints, small depressions, large surface debris, and other unusual micro-features were avoided during sampling.

Soil cores and bulk soil samples used for determination of denitrification, net N_2O emission rates, microbial biomass C and N and other relevant physico-chemical properties were collected on May 19, 20, 30, and June 18, 2005 from the LF, NF, HF, and CF sites respectively. To avoid high initial soil NO_3 concentration, cores from the LF and CF sites were collected on dates when no nursery runoff was entering the sampling plots. At each sampling plot, 9 intact soil cores (6 cm dia. x 10 cm length) were collected in plastic liners (6 cm dia. x 15 cm length) using a slide hammer (AMS core sampler®, American Falls, Idaho). The collected cores were capped at both ends. An additional soil core (0-10 cm soil depth) was collected from each plot in bronze liners (6 cm dia. x 10 cm length) for determination of bulk density and moisture content. Finally, 4 soil cores (0-10 cm soil depth) were collected and composited using a mud auger (4.4 cm dia.) for analysis of physico-chemical properties, a potential denitrification enzyme assay, and concentrations of nitrate and ammonium. The % water-filled pore space (WFPS) of all the cores collected from the LF, NF, CF and HF sites was 100, 100, 80 and 83%, respectively, at the time of sampling. The %WFPS of the soil samples were determined according to Ullah et al. (2005). The intact cores and bulk soil samples were transferred to the laboratory on ice and refrigerated until use.

Soil cores used for potential net N mineralization and nitrification rates were collected from all sampling plots during the last week of October, 2005. Duplicate, intact soil cores (10 cm long) were obtained as described above and transferred to the laboratory on ice, where they were refrigerated until use.

Potential denitrification assay

Potential denitrification was determined using soil slurries according to Hunter and Faulkner (2001). Field moist soils (10 g dry-soil weight basis) were weighed into four 150 ml serum bottles from each bulk soil sample and were assigned randomly to one of the four treatments – unamended control, 5 µg PO_4 g^{-1} soil, 15 µg NO_3-N g^{-1} soil, and 15 µg NO_3-N +5 ug PO_4 g^{-1} soil in a factorial design. For each treatment 4 replicates were used. After weighing soils in serum bottles, 10 ml of PO_4 solution delivering 5 µg PO_4 g^{-1} soil (as KH_2PO_4) was added to 4 bottles each labeled as PO_4 only and PO_4 + NO_3.

The remaining 8 bottles received 10 ml of DI water. The bottles were closed with rubber stoppers and shaken for 10 minutes to make slurry. After shaking, the rubber stoppers were removed and the bottles were wrapped in aluminum foil and allowed to equilibrate for 48 hours. It was assumed that 48 hours duration would be sufficient to expose microbes in the slurry to the added PO_4 for cellular incorporation, keeping in mind the rapid turnover (in the order of hours) and assimilation of PO_4 by the phosphate accumulating microbes in the soil (Meyer et al. 2005).

After 48 hours, 10 ml of a NO_3 solution (as KNO_3) was administered to 4 bottles each labeled as NO_3 only and PO_4 + NO_3 treatments, while 10 ml DI water was added to the remaining 8 bottles. Bottles were then capped using serum septa and purged with O_2-free N_2 gas for 25 minutes to induce anaerobic conditions. After purging, 10% of the headspace was replaced with acetylene (C_2H_2) gas that had been purified in concentrated H_2SO_4 solution and DI water sequentially for the removal of acetone. After the addition of C_2H_2, the bottles were wrapped in aluminum foil and shaken continuously for 6 hours on a reciprocating shaker at room temperature (appx. 22 ^oC). Headspace gas samples (9 ml) were collected from the bottles after 0 and 6 hours using a hypodermic needle attached to a syringe. The gas samples were injected into 5 ml Becton Dickinson Vacutainers to maintain a high internal pressure to avoid any diffusion of outside air into the Vacutainers. The gas samples were analyzed within one week of collection on a Shimadzu GC-14A gas chromatograph equipped with an electron capture detector. The rate of N_2O production, determined from the rate of accumulation of N_2O in the headspaces of the bottles, was corrected for dissolved N_2O in the slurry using the Bunsen absorption coefficient of 0.54 (Tiedje 1982). Denitrification potential was converted to an area basis (while accounting for differences in bulk density of the four sites) and is reported as µg N m^{-2} h^{-1}.

Denitrification and net N_2O emission rates from soil cores

Denitrification and net N_2O emission rates were determined on intact soil cores brought to room temperature and incubated for 24 hours. The purpose was to quantify the response of these soils in terms of denitrification and net N_2O emissions within the first 24 hours of NO_3 loading. The 24 hours duration was chosen to simulate a hydrologic retention time of 24 hours of the loaded NO_3 into the riparian soils due to runoff. The 9 cores collected from each sampling plot were randomly assigned to groups of three cores each. One set was randomly selected for measuring net N_2O flux while the remaining 2 sets were prepared for measuring denitrification rate with and without an added PO_4 amendment The set to receive additional PO_4 was amended with a 5 ml phosphorus solution to deliver 5 µg PO_4 g^{-1} soil, while the remaining cores received 5 ml DI water. All sets of cores were covered and equilibrated for 48 hours to give sufficient time for microbes in the PO_4 amended treatment to be exposed to the added PO_4. After 48 hours, a 5 ml solution containing 0, 30, or 60 µg NO_3-N g^{-1} was administered to one core within each set.. A syringe was used to evenly distribute the NO_3 solution to the surface of the core. The WFPS of each core was brought to 100% by adding DI water to the cores where WFPS was less than 100%. This was done to simulate a sudden increase in NO_3 loading of the riparian soil under saturated soil conditions, delivered by nursery runoff after an irrigation or rainfall event. After amendment with NO_3, purified C_2H_2 gas was injected into the two sets of cores selected for determination of denitrification rate. Approximately 10 ml C_2H_2 gas was injected directly into the cores at the liner and soil column interface in small aliquots using a syringe fitted with a 16 gauge 10-cm long needle. This was done to ensure a rapid and even diffusion of C_2H_2 gas into the soil pore space. The purpose of injection of C_2H_2 at the liner and soil column interface instead of the middle of the columns was to avoid disturbance to the soil column. After C_2H_2 injection, the cores were sealed with airtight seals fitted with rubber septa for gas sampling. The headspace in the closed column was replaced with an additional 5 ml C_2H_2 gas to achieve an approximate 10% C_2H_2 gas concentration in the column. The last set of cores selected for net N_2O emission were sealed with airtight caps without the addition of C_2H_2 gas. Soil cores incubated with and without additional C_2H_2 gas were used to estimate denitrification and net N_2O emission rates. Gas samples, collected after 0 and 24 hours of incubation from the closed column headspace using a syringe, were analyzed on a gas chromatograph for concentration of N_2O as described in the previous section. The rates of denitrification and net N_2O emissions determined are reported as µg N m^{-2} h^{-1}.

Microbial biomass carbon and nitrogen

Bulk soil samples collected from the four sites were used for the determination of microbial biomass C according to Voroney et al. (1993). Four replicate (25 g field-moist soils) soil samples were fumigated in a desiccator for 24 hours to kill and lyse microbial cells in the soil. The fumigated and a similar set of non fumigated soils (4 replicates each for each forest site) were extracted with 0.5 M K_2SO_4 solution for soluble organic carbon (C) concentration at 1:8 soil to K_2SO_4 solution ratio . The extracts were filtered through No. 42 Whatman filter paper into 20 ml vials and analyzed using a Shimadzu TOC analyzer for determination of soluble organic C. Before analysis, samples were diluted by a factor of 4 to reduce the concentration of K_2SO_4 salts in the extracted samples because salt passing through the TOC analyzer can clog the beaded column. The amount of microbial biomass C was calculated as the difference of soluble organic C between fumigated and unfumigated soils divided it by a correction factor (K_{EC} = 0.40) to account for the efficiency of fumigation-extraction of the microbial C. Microbial biomass N was determined using the chloroform fumigation-incubation technique according to Voroney and Paul (1984). Four replicate (25g field-moist soils) samples from each forest site were fumigated in a desiccator for 24 hours as described above. The fumigated samples were inoculated with fresh soil for 10 days at room temperature ((~22 $^{\circ}$C) to allow mineralization of organic N in the sample including that in the lysed microbial cells. A similar set of non fumigated samples (4 replicates for each forest site) were also incubated with the fumigated samples. After the 10 days incubation, the samples were extracted with 2M KCL for mineral N concentration determination. Microbial biomass N was calculated as the difference in mineral N in fumigated and non fumigated soils divided by a correction factor (K_{EN} =0.30) to account for the efficiency of microbial N extraction. Both the microbial biomass carbon and nitrogen are reported as µg C or N g^{-1} dry soil.

Selected physico-chemical properties of soils

Gravimetric soil moisture content, bulk density, total porosity, water-filled pore space, soil particle size distribution, soil pH, mineral nitrogen, water-soluble organic carbon, and total soil C and N were determined on bulk soil samples according to Ullah et al. (2005). Total soil P content was determined using Mehlich 3 method of soil extractable nutrients.

Potential net N mineralization and nitrification rates

One of the duplicate soil cores from each sampling plot collected in October, 2005 was homogenized thoroughly by hand, and a 5 g sub-sample was extracted with 2 M KCL solution for the determination of initial mineral N concentration. The WFPS of the remaining soil cores was adjusted to 100% by adding DI water to the top of the cores. The cores were covered with a loose cap to allow for air exchange and to reduce the loss of water vapor and were then placed in a box to incubate in the dark at 20 $^{\circ}$C for 28 days (Hart et al. 1994). These cores were incubated at 100% WFPS to simulate conditions similar to the cores incubated for the determination of denitrification rates. Following the incubation period, the cores were removed from the plastic liners and homogenized thoroughly by hand. A 5 g sub-sample of the homogenized soil was extracted with 2 M KCL solution for the determination of mineral N. Net nitrogen mineralization and nitrification rates were calculated from the difference in the amount of initial and final mineral N content (Hart et al. 1994). Net nitrogen mineralization and nitrification rates, are reported as ng N g^{-1} dry soil h^{-1}.

Foliar Nitrogen

Eight replicate samples of fresh leaf litter were collected from each 1 m^2 plots at the four forest sites on October 30, 2005. The samples were oven-dried at 65 $^{\circ}$C for 5 days. The dried samples were pulverized and analyzed on a LECO N analyzer using a thermoconductivity detector for the determination of foliar N, which is reported as % N on dried mass basis (Table 1).

Statistical Analysis

All data were analyzed using SAS V-8.3 (SAS Inc. 2000). Within-site differences in denitrification and net N_2O emission rates of soils amended at 0, 30, and 60 µg NO_3 g^{-1} soil were done using analysis of variance (ANOVA) using the General Linear Model. Fisher's protected LSD was used for post hoc comparisons at $\alpha = 0.05$. Similarly, ANOVA was also used for between-site comparison of denitrification , net N_2O emission and N mineralization and nitrification rates. To elucidate any effect of PO_4 amendment on denitrification rate, a two-sample T test was done using the pooled variance technique at $\alpha = 0.05$. A multiple regression model using the backward-selection option was used to identify predictor variables that significantly affect denitrification and net N_2O emission rates from the selected sites. The data was analyzed to meet the normal distribution assumption of ANOVA and regression using the Proc Univariate procedure at Shapiro-Wilk significance of $p > 0.05$. Pearson correlation coefficients between various microbial and physio-chemical characteristics of the sites were determined using SAS.

Results

Potential denitrification assay

The potential denitrification rate of riparian soils either exposed or not exposed to mineral N loading from nursery runoff increased significantly ($p < 0.05$) when amended with 15µg NO_3 g^{-1} soil alone or in combination with PO_4 (Figure 1). The addition of PO_4 had no effect on potential denitrification in soils from any of the sites. A significant response of these soils to added NO_3 in terms of increased denitrification depicts a limitation of this process by available NO_3 even after prolonged exposure of the LF and CF sites to mineral N loading.

Denitrification and net N_2O emission rates from soil cores

When intact soil cores were amended with 30 µg NO_3 g^{-1} soil, samples from all the sites responded with a significant increase in denitrification rate compared to non amended soils (Table 2), showing that denitrification in these sites is limited by NO_3 in a manner similar to that found in Figure 1. The denitrification rates observed among sites amended with 30 µg NO_3 g^{-1}, however, did not significantly differ ($p > 0.05$). Although denitrification rate was further increased in soils amended with 60 µg NO_3 g^{-1}, this was not significant except in soil from the NF site. The addition of 5 µg PO_4 g^{-1} soil made little difference in denitrification rate (Table 3

The addition of 30 µg NO_3 g^{-1} soil to soil cores collected from all riparian sites increased net N_2O emissions by an average of 15-fold compared to the unamended treatment (Table 4). However, N_2O emission rates averaged from soils collected from the N-exposed sites (22.5 µg N m^{-2} h^{-1}) were 1.5 times those of the non-exposed sites (14.5 µg N m^{-2} h^{-1}) at 30 µg NO_3 g^{-1} amendment level. With 60 µg g^{-1} additional NO_3, net N_2O emissions increased significantly ($p < 0.05$) compared to the 30 µg NO_3 g^{-1} treatment in soils from the N-exposed sites. Moreover, N_2O emission rates from the N exposed sites were on average 1.6 times higher ($p < 0.05$) than N_2O emission rates from the non-exposed sites (Table 4).

Soluble organic carbon (SOC) was a key predictor variable of denitrification (multiple linear regression) in soils from the four riparian forest sites when amended with 30 and 60 µg NO_3 g^{-1} soil, respectively (Figures 2 and 3). SOC accounted for 30% of the variability in denitrification rate (denitrification in µg N m^{-2} h^{-1} = 294 + 0.58 SOC in µg C g^{-1} soil) for the 30 µg NO_3 g^{-1} treatment, whereas this factor accounted for only 55% of the variability at the 60 µg NO_3 g^{-1} amendment level (denitrification in µg N m^{-2} h^{-1}= 199 + 1.70 SOC in ug C g^{-1} soil). SOC controls denitrification rates in these sites once the process is not limited by NO_3 availability. Unlike denitrification, no single strong predictor variable of N_2O flux from these forests was identified due to greater variability of the flux rates and the complex interactions of the predictor variables in regulating the flux- a condition encountered by other researchers (Smith et al. 1995; Groffman, et al. 2000b). The combination of various predictor variables accounted for 93%, 48% and 83% variability in net N_2O emissions at zero, 30 and 60 µg NO_3 g^{-1} amendment levels, respectively. Among these variables, microbial biomass nitrogen, total soil nitrogen and NH_4 concentration correlated positively with net N_2O emissions in the regression models. This suggests that an increases in different pools of soil nitrogen due to chronic N loading can increase N_2O emissions during denitrification.

Microbial biomass carbon and nitrogen

Compared to soils from sites exposed to nursery runoff, relatively higher soil C:N ratio and microbial biomass C in the soils from sites not exposed to nursery runoff (Table 1) indicates a higher pool of labile C available to denitrifiers, resulting in higher denitrification and lower net N_2O emission rate. Microbial biomass carbon, SOC, and total soil C correlated significantly with denitrification rate, whereas microbial biomass N, total soil N, NH_4, and C:N ratios correlated significantly with net N_2O emission (Table 5).

Potential net N mineralization and nitrification rates

Potential net nitrogen mineralization rates were not significantly different in soils collected from the four riparian forest sites ($p > 0.05$). Potential net nitrification rate, however, differed significantly ($p < 0.05$) between N-exposed and non-exposed sites (Table 1). The N-exposed sites had 8.4 times higher nitrification rates than those observed in the non-exposed sites. Total foliar nitrogen content was 1.2 times higher in leaf litter collected from sample plots on the N-exposed sites than litter collected from non-exposed sites (Table 1).

Discussion

Denitrification rate in soils collected from riparian forest sites either exposed or not exposed to mineral N loading, increased significantly in all the sites when amended with NO_3. This observation clearly demonstrates that denitrification in soils from these sites was limited by NO_3 (Figure 1; Tables 2 and 3) and that prolonged mineral N loading did not affect the activity of denitrifying microbes in the soils collected from exposed sites (LF and CF sites). Hanson et al. (1994a and 1994b) also observed higher denitrification rates in a N-enriched riparian forest in Rhode Island, and they concluded that higher denitrification capacity is a key process that moderates the effects of chronic mineral N enrichment. Average lower soil NO_3 (Table 1) concentration (2.9 µg N g^{-1} soil) in the N-exposed sites in spite of chronic run-off input support the observation that NO_3 removal capacity of these sites is not exhausted by chronic N loading. In a study in Europe, lower NO_3 concentrations in groundwater beneath a riparian forest receiving chronic N run-off was ascribed to higher denitrification rates (Hefting and de Klein 1998), which is in agreement with our results.

The observed rates of denitrification (Tables 2 and 3) in soils from all sites were within the range of denitrification rates in riparian forest soils reported elsewhere in literature (Lowrance et al. 1995; Jordan et al. 1998; Hefting et al. 1998 and 2003). However, caution needs to be exercised when extrapolating denitrification rates of the current study to bigger spatial and temporal scales, since these rates were determined under controlled laboratory conditions of soil NO_3, temperature and moisture and thus may not reflect actual field conditions.

As the addition of NO_3 to soil cores increased denitrification, the rate limiting factor shifted from NO_3 availability to available organic C substrate, especially at 60 µg NO_3 g^{-1} soil treatment. For example, soil from the non-exposed NF site with significantly higher SOC and total soil C (Table 1) denitrified more NO_3 than the rest of the sites at 60 µg NO_3 g^{-1} amendment level. This apparent control of denitrification rates by available C substrate was found significant using the multiple regression and Pearson's correlation analyses (Figures 2 and 3; Table 5). Significant control of denitrification rates by available C substrate in riparian wetlands has been reported elsewhere in the literature (Lindau, et al. 1994; Lowrance, et al. 1995; DeLaune et al. 1996; Hefting et al. 2003).

Microbial biomass C also correlated significantly with denitrification rates (Table 5) supporting the argument that available C exerts a regulatory control on denitrification rate, as biomass C is one of the sources of the labile C pools in soil. However, it is noteworthy that the microbial biomass carbon content (Table 1) of the N-exposed sites was significantly lower than those of the non-exposed sites ($p < 0.05$). Lower microbial biomass C in the N-exposed sites is thought to be due to the negative effects of prolonged N exposure. This finding is in agreement with those of Compton et al. (2004), Bowden et al. (2004) and Wallenstein et al. (2006), who observed lower microbial biomass carbon and activity in N-enriched temperate forest soils in the northeastern U.S. Wallenstein et al. (2006) also reported a 59 and 52% reduction in microbial biomass C and substrate-induced respiration, respectively, in soils of a N-saturated temperate forest compared to a non-saturated forest in New England. Ettema et al. (1999) observed similar effects of N enrichment on biomass C and activity in riparian forest soils in Georgia. These authors feared that the denitrifying microbes in riparian forests may be threatened by the cumulative negative effects of N saturation. Although we found significantly lower soil microbial biomass C in the N-exposed sites, the current study did not observe significant differences in denitrification rates among the N-exposed and non-exposed sites, showing that riparian forests can sustain a high and persistent capacity to denitrify NO_3 even if exposed to prolonged mineral N loading (Hanson et al. 1994b). Given the limited temporal coverage of this experiment under optimum laboratory soil moisture and temperature regimes, further temporally intensive field denitrification assessment studies of these sites is recommended to validate the current observations.

We found no effect of PO_4 addition on denitrifier activity (Figure 1; Tables 2 and 3), which is commensurate with the results of Federer and Klemedtsson (1988) and White and Reddy (1999). However, our findings are in contrast to those of Sudareshwar et al. (2003) who reported that P-enrichment of coastal wetland soils reduced denitrification potential compared to similar non-enriched soils. None of these studies were conducted on riparian forest soils. Our data suggests that P input to riparian forests from agricultural run-off will not affect denitrifier activity.

Even though denitrification rate in soils amended with additional NO_3 (30 and 60 µg NO_3 g^{-1}) varied little among sites (Table 2), net N_2O emission rates were higher from soils collected from the N-exposed sites (Table 4). It appears that these differences were a result of prolonged exposure of the N-exposed sites to nursery run-off. This result is consistent with the findings of Hefting et al (2003) who reported that N_2O emissions from riparian forests receiving chronic N loads were higher compared to emissions from riparian grasslands, even though denitrification rates of the two ecosystems were similar. Higher soil N pools, greater potential nitrification rates, and lower soil and microbial biomass C:N ratios (Table 1) resulting from prolonged N loading in the N-exposed soils appeared to have reduced soil N_2O reductase activity, which eventually led to higher N_2O emissions compared to emissions from the non-exposed sites. Moreover, prolonged N exposure resulted in higher nitrification rates in the N-exposed sites (Magill et al. 2000) compared to the non-exposed sites. This observation is similar to those in other studies that evaluated N_2O emissions from temperate forest soils after N fertilization in the northeastern U.S. (Bowden et al. 1991; Brumme and Beese 1992; Sitaula and Bakken, 1993; Barnard et al. 2005).

In findings similar to ours, Hanson et al. (1994b) reported significantly higher microbial biomass N in a N-enriched riparian forest soil compared to a non-enriched site (Hanson et al. 1994b), suggesting that prolonged exposure of riparian forests to mineral N is saturating different soil N pools. The soil N saturation phenomena, including increases in microbial biomass N and net nitrification rates, may be resulting in relatively higher N_2O emissions from riparian forests when loaded with mineral N from agricultural run-off. Although a significant relationship (r = 0.50; $p < 0.04$) found between microbial biomass N and N_2O emissions from cores amended with 60 µg NO_3-N g^{-1} soil (Table 5), this does not likely represent a cause and effect relationship. Further studies are needed to define the relationship between an increase in microbial biomass N and higher N_2O emissions in riparian forest soils.

In this study, microbial biomass C was significantly lower ($p < 0.05$) in the N-exposed sites (Table 1) compared to the non-exposed sites, which is in agreement with the findings of Ettema et al. (1999), Bowden et al. (2004), and Compton et al. (2004). Concomitant decrease in biomass C with increasing biomass N and increased net nitrification rates due to prolonged exposure of riparian forests to mineral N loading strongly suggests that episodic, high levels of NO_3 input into N-saturated riparian forest soil leads to higher net N_2O emissions.

Soil texture affects N_2O flux from soils by influencing gas diffusion rates in the soil profile (Weitz et al. 2001). Compared to coarse-textured soils, fine-textured soils limit gas diffusion rates, thus enhancing the probability that N_2O is reduced to N_2 gas by soil denitrifying organisms (Weitz et al. 2001). Although the N-exposed sites (CF and LF) were higher in clay (Table 1), net N_2O emissions from these soils exceeded those of sites not exposed to additional mineral N loading, supporting our finding that that prolonged exposure of riparian forest soils to mineral N may have reduced N_2O reductase activity. Soil water can also reduce N_2O diffusion by approximately 4 orders of magnitude by filling and blocking up soil air pores. This increases the time for microbial reduction of N_2O to N_2 gas before its emission into the air (Clough et al. 2005). Saturated soil conditions of the soil cores at the time of incubation may have obscured the effect of soil texture on N_2O emissions from the four sites. We recommend further studies to elucidate the interactive effects of soil moisture and texture on N_2O emission from soils to better understand the fate of N_2O in soils.

In our study, N_2O emission rates in treatments that did not receive additional NO_3 were within the range or lower than the N_2O emission rates reported by other studies from temperate forests in the northeastern U.S. (Bowden et al. 1990, 1991, 2000; Hafner and Groffman 2005). However, when additional NO_3 is loaded into riparian forests, which are considered as 'hotspots' of denitrification and N_2O production (Groffman et al. 2000a), N_2O emission rate increases by a factor of at least 12 or more even under saturated soil conditions. The increase in N_2O emissions due to NO_3 loading needs to be considered when calculating N_2O emission factors for riparian forests by concerned agencies (Groffman et al. 2000a) like the Intergovernmental Panel on Climate Change and the U.S. Department of Energy-National Commission on Carbon Sequestration.

In summary, the results of this research show that the denitrification potential of riparian forest soils is not compromised after chronic exposure to mineral N run-off for 10 years. Moreover, addition of PO_4 does not seem to affect the activity of denitrifying microbes in these soils. Although riparian soils can substantially contribute to the reduction of NO_3 loading into water bodies in watersheds dominated by plant nurseries, these forests will emit relatively more N_2O into the atmosphere compared to similar soils not exposed to chronic mineral N run-off. This should be accounted for at the landscape scale within the wetlands potential carbon-sequestration context. We recommend that riparian forests be considered as an integral component in developing strategies for NO_3 removal from nursery run-off in New Jersey and other similar eco-zones in the country.

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List of Figures

Table 1. Selected soil (0-10cm depth) properties of riparian forest sites exposed to mineral N loading from nursery runoff (mean ± standard error)

Soil properties	**N Exposed sites**		**Non-exposed sites**	
	LF	**CF**	**NF**	**HF**
Clay (%)	39 ± 1.7	33 ± 7	8 ± 1	23 ± 1.5
Silt (%)	51 ± 1.3	29 ± 3	9 ± 1	54 ± 9
Soil texture	Silty clay loam	Clay loam	Loamy sand(organic)	Silt loam
Approximate area (acres)	5	15	10	5
Bulk density (g cm^{-3})	0.90 ± 0.16	0.96 ± 0.07	0.46 ± .03	1.05 ± .05
Porosity (cm^3 cm^{-3})	0.61 ± 0.06	0.63 ± 0.02	0.82 ± 0.01	0.60 ± .02
Water-filled pore space (%)	100 ± 27	80 ± 4	100 ± 0.20	83 ± 12
pH	6.3 ± 0.1	5.4 ± 0.2	4 ± 0.1	5.7± 0.2
Soluble organic C (µg g^{-1})	108 ± 5	163 ± 18	300 ± 32	158 ± 15
Microbial biomass C (µg g^{-1})	713 ± 65	978 ± 94	2578 ± 351	1238 ± 132
Microbial biomass N (µg g^{-1})	394 ± 70	383 ± 75	315 ± 54	165 ± 29
Total P (µg g^{-1})	177 ± 4	222 ± 36	27 ± 13	87 ± 26
NO_3-N (µg N g^{-1})	2.7 ± 1.8	3.1 ± 0.6	0.92 ± 0.32	1.9 ± 1.16
NH_4-N (µg N g^{-1})	41 ± 5	23 ± 2	14 ± 1	8 ± 1
Total C (% of dry soil)	4.6 ± 0.60	3.7 ± 0.50	8.3 ± 0.64	3.9 ± 0.20
Total N (% of dry soil)	0.37 ± 0.03	0.23 ± 0.03	0.38 ± 0.03	0.20 ± 0.01
C:N ratio	12.1	16.0	22.0	19.0
N mineralization rate (µg N g^{-1} h^{-1})	74 ± 28	91 ± 7	156 ±79	98 ± 45
Nitrification rate (µg N g^{-1} h^{-1})	18 ± 6.1	41 ± 8.4	4 ± 1.2	3 ± 0.9
Foliar N (% mass basis)	1.36 ± 0.11	1.32 ± 0.08	1.11 ± 0.6	1.11 ± 0.11

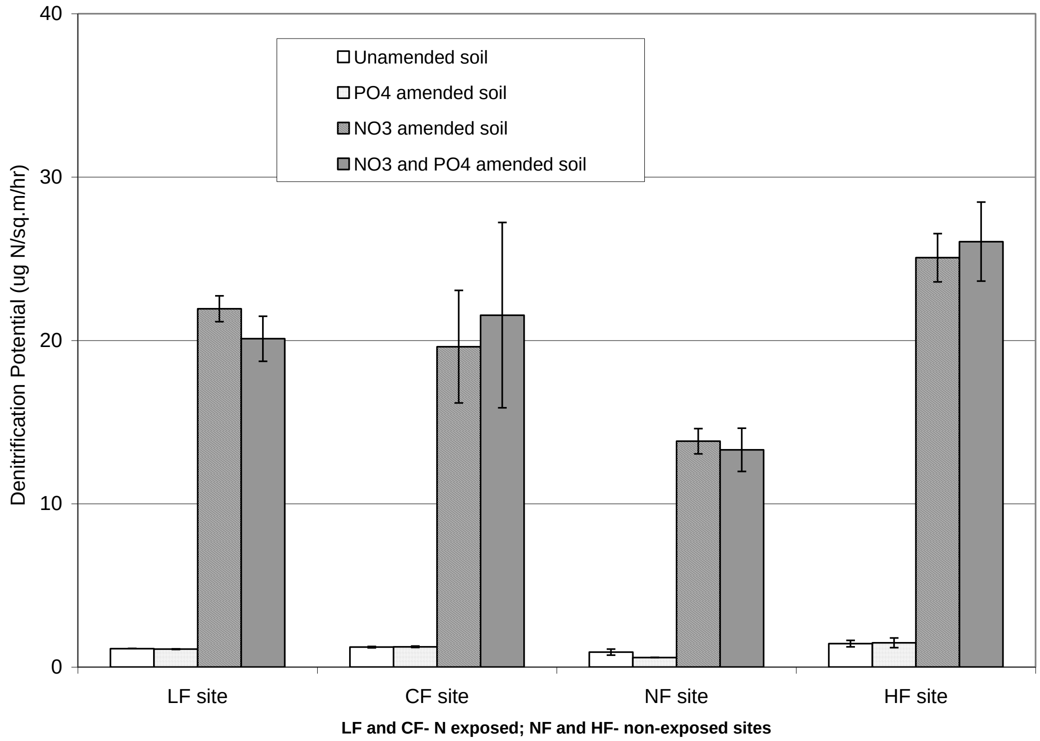

Figure 1. Mean potential denitrification rate and standard error of soil slurries from riparian forest soils exposed (LF, CF) or not exposed (NF, HF) to mineral N loading from nursery runoff.

Table 2. Denitrification rate (mean ± standard error) of soil from riparian sites exposed (LF, CF) or not exposed (NF, HF) to N from nursery runoff.

Additional NO_3 (μg NO_3 g^{-1})	**N exposed sites**		**Non-exposed sites**	
	LF	**CF**	**NF**	**HF**
	…………………..Denitrification rate (μg N m^{-2} h^{-1})…………………			
0	163 ± 30 a[a]	136 ± 35 a	147 ± 09 a	150 ± 26 a
30	362 ± 55 b	431 ± 28 b	458 ± 21 b	346 ± 45 b
60	398 ± 76 b	474 ± 105 b	674 ± 104 c	515 ± 80 b

[a] Means followed by same letters in a column show no significant difference ($p > 0.05$) using an ANOVA test.

Table 3. Denitrification rate (mean ± standard error) of soil from riparian sites exposed (LF, CF) or not exposed (NF, HF) to N from nursery runoff and amended with 5 µg PO_4 g^{-1} soil.

Additional NO_3 (µg NO_3 g^{-1})	**N exposed sites**		**Non-exposed sites**	
	LF	**CF**	**NF**	**HF**
	………….Denitrification rate (µg N m^{-2} h^{-1})………….			
0	152 ± 23 a[a]	152 ± 35 a	90 ± 12 a	97 ± 34 a
30	351 ± 56 b	424 ± 28 b	425 ± 35 b	357 ± 60 b
60	451 ± 37 b	505 ± 105 b	625 ± 37 c	459 ± 64 b

[a] Means followed by same letters in a column show no significant difference ($p > 0.05$) using an ANOVA test.

Table 4. Net N_2O emission rates (mean ± standard error) of soil from riparian sites exposed (LF, CF) or not exposed (NF, HF) to N from nursery runoff.

Additional	**N exposed sites**		**Non-exposed sites**	
NO_3 (µg NO_3 g^{-1})	**LF**	**CF**	**NF**	**HF**
	………………**Net N_2O emission rate (µg N m^{-2} h^{-1})**…………………			
0	3 ± 0.6 a	1 ± 1.3 a	1.20 ± 0.5 a	0.8 ± 0.9 a
30	25 ± 1.8 b	20 ± 2.7 b	17 ± 4.7 b	12 ± 2.1 b
60	33 ± 2.7 c	32 ± 3.1 c	22 ± 2.2 b	17 ± 2.8 b

[a] Means followed by same letters in a column show no significant difference ($p > 0.05$) using an ANOVA test.

Table 5. Relationship between denitrification rate and N_2O emission rate to various soil factors (Pearson correlation analysis) in riparian forest soils amended with 0, 30, and 60 µg NO_3 g^{-1} soil.

	Denitrification rate (µg N m^{-2} h^{-1})			**..N_2O emission rate (µg N m^{-2} h^{-1})..**		
Additional NO_3 g^{-1} soil	**0**	**30**	**60**	**0**	**30**	**60**
Variables						
Soluble organic C	0.07[a] (0.78)[b]	0.55* (0.02)	0.74* (.0009)	-0.41 (0.10)	-0.10 (0.70)	0.15 (0.55)
Microbial biomass C	0.08 (0.77)	0.54* (0.03)	0.72* (0.001)	-0.38 (0.14)	0.16 (0.53)	0.10 (0.69)
Microbial biomass N	-0.15 (0.57)	0.20 (0.45)	-0.35 (0.18)	-0.01 (0.94)	0.38 (0.14)	0.50* (0.04)
Total C	0.08 (0.75)	0.26 (0.32)	0.46** (0.07)	-0.06 (0.82)	0.11 (0.67)	-0.04 (0.86)
Total N	0.20 (0.44)	0.11 (0.67)	0.16 (0.54)	0.55* (0.02)	0.24 (0.35)	0.11 (0.66)
C:N ratio	-0.10 (0.66)	0.22 (0.40)	0.48 (0.05)	-0.72* (0.001)	-0.16 (0.54)	0.10 (0.70)
pH	-0.02 (0.92)	-0.41 (0.11)	-0.52* (0.04)	0.59* (0.01)	0.02 (0.92)	-0.18 (0.48)
Total P	-0.22 (0.42)	-0.08 (0.76)	-0.48** (0.06)	0.29 (0.29)	0.43 (0.10)	0.41 (0.12)
NO_3	0.22 (0.40)	-0.29 (0.26)	-0.43 (0.09)	0.15 (0.57)	0.06 (0.80)	0.25 (0.34)
NH_4	-0.04 (0.85)	0.05 (0.83)	-0.35 (0.18)	0.84* (0.0001)	0.22 (0.40)	0.02 (0.92)

[a] Pearson correlation coefficient.

[b] Significance (n =16) at $p < 0.05$ (*), $p < 0.10$ (**), or not significant (no asterisk).

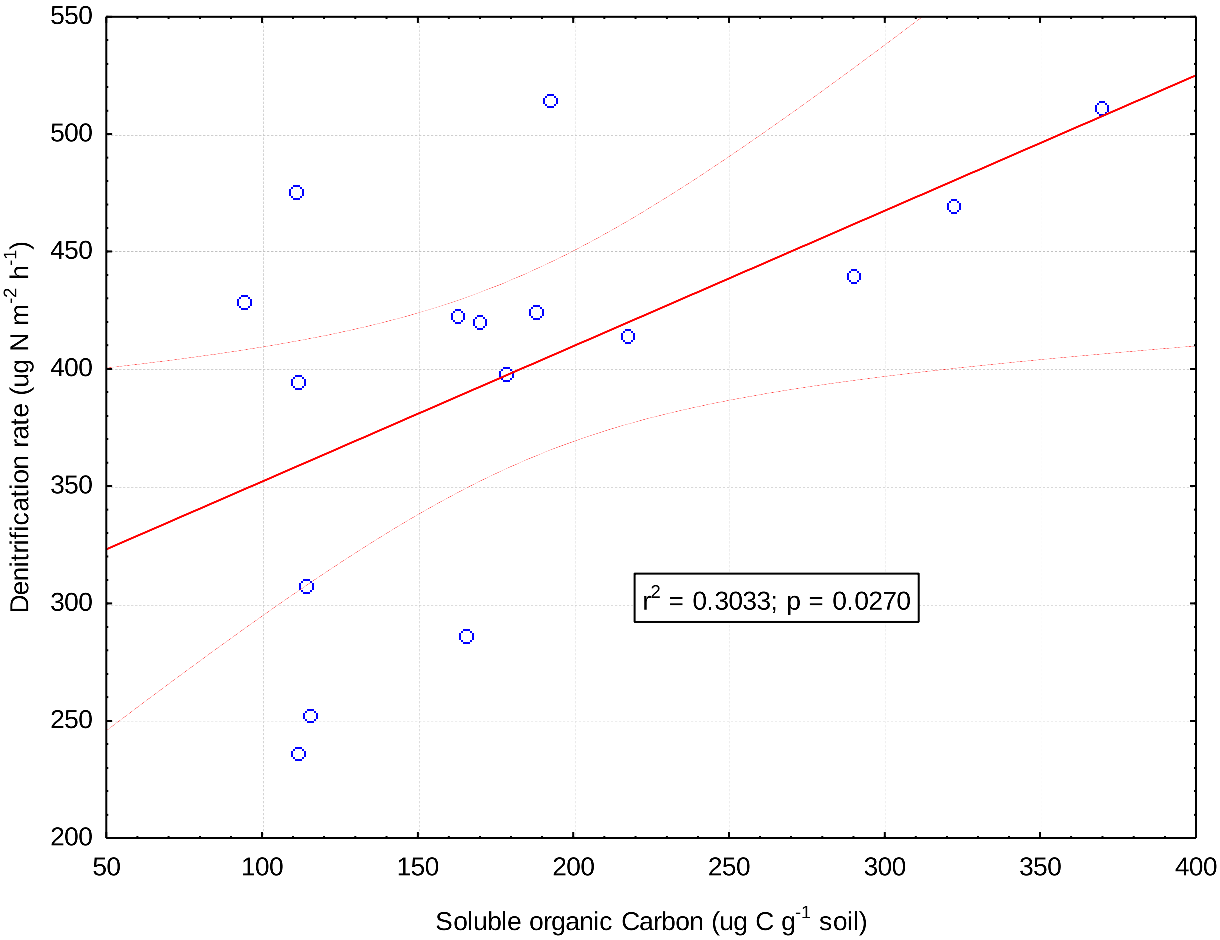

Figure 2. Relationship between denitrification rate and soluble organic carbon in soils from riparian forest soils amended with 30 µg NO_3 g^{-1} soil (Y = 294 + 0.58 X).

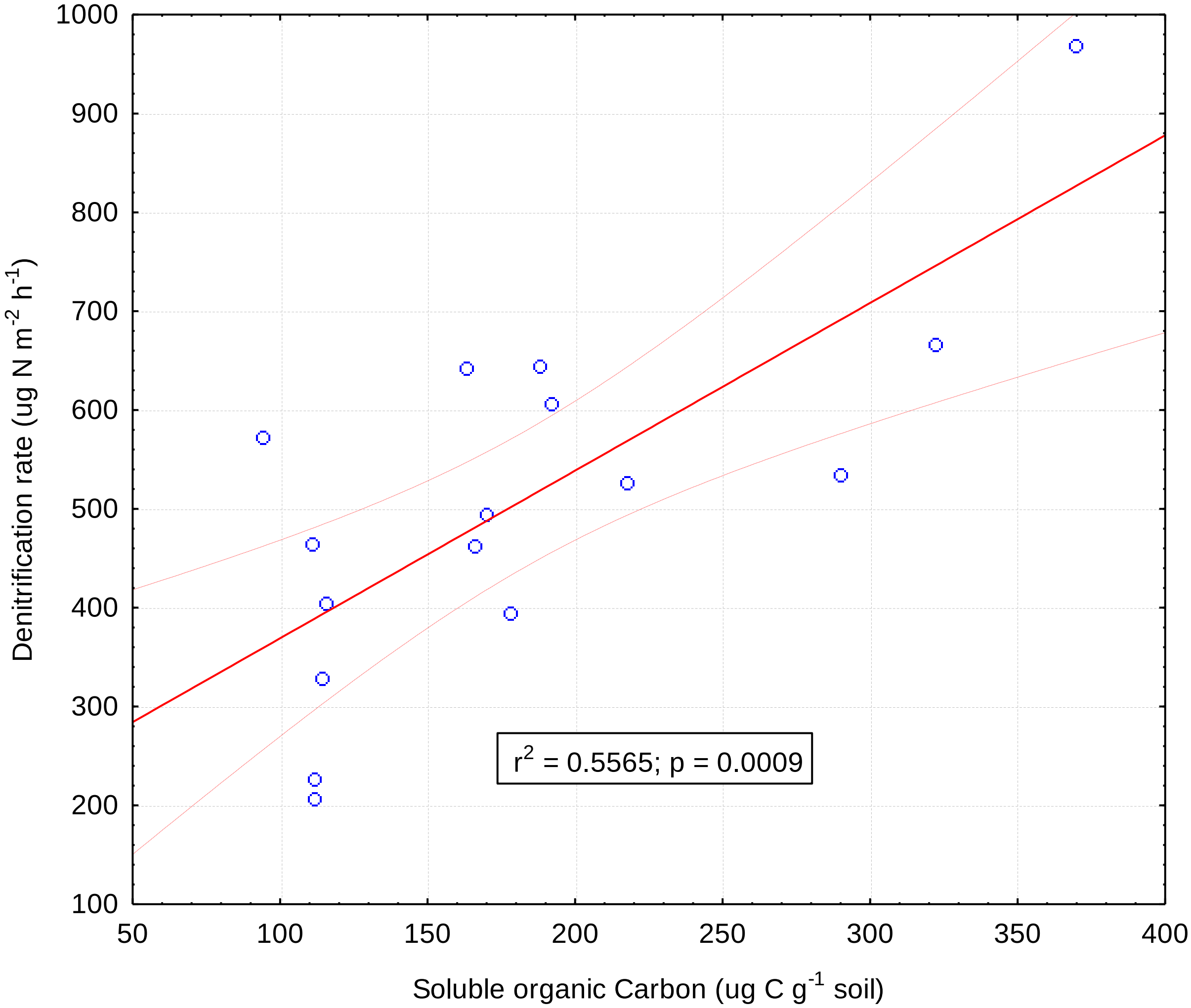

Figure 3. Relationship between denitrification rate and soluble organic carbon in soils from riparian forest soils amended with 60 µg NO_3 g^{-1} soil (Y = 199 +1.70 X).