WATER QUALITY EFFECTS OF SWITCHGRASS INTERCROPPING ON PINE FOREST IN COASTAL NORTH CAROLINA

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ABSTRACT. *Interplanting a cellulosic bioenergy crop (switchgrass,* Panicum virgatum *L.) between loblolly pine (*Pinus taeda *L.) rows could potentially provide a sustainable source of bio-feedstock without competing for land currently in food production. The objectives of this study were to: (1) quantify the concentrations and loads of drainage water nitrogen (N) and phosphorus (phosphate) associated with establishment and growth of switchgrass treatments and compare them with those for a mid-rotation pine forest (control), and (2) quantify the treatment effects on drainage water N and phosphate and compare the effects between treatments, i.e., switchgrass intercropped into young loblolly pine (IC) and switchgrass only (SG). Thinned mid-rotation loblolly pine with natural understory (MP) was used as the control. Pretreatment calibration equations for nutrients were obtained using a paired watershed approach and bootstrap geometric regression with 2007-2008 data, when pine on all sites had reached canopy closure. Treatment effects were calculated as the difference between expected values from the pretreatment relationship and measured data for the treatment period. Precipitation, outflow, and N and phosphate concentrations in the outflow were measured during calibration (Jan. 2007 to Dec. 2008), site preparation for switchgrass establishment (Nov. 2009 to Mar. 2012), and switchgrass growth (Apr. 2012 to Apr. 2014). Mean NO_3-N concentrations and loads were significantly (α = 0.05) greater for SG than for IC during the switchgrass growth period. Average treatment concentrations with standard errors and total load effects during switchgrass growth for NO_3-N followed the trends SG (-0.002 ±0.01 mg L^{-1}) > IC (-0.12 ±0.04 mg L^{-1}) and SG (0.75kg ha^{-1}) > IC (0.23kg ha^{-1}), respectively. For phosphate average concentrations and loads, the treatment effects during switchgrass growth followed the trends SG (-0.004 mg L^{-1}) >IC (-0.02 mg L^{-1}) and IC (-0.43 kg ha^{-1}) > SG (-0.70 kg ha^{-1}), respectively. Average concentration effects for NO_3-N and phosphate and total load effects for phosphate significantly (α = 0.05) decreased for IC compared to the MP control. These results suggest that the intercropping treatment (IC) with loblolly pine and switchgrass improved water quality by reducing NO_3-N and phosphate concentrations and phosphate loads.*

Keywords. *Bioenergy crop, Bootstrap geometric regression, Loblolly pine, Nutrients, Paired watershed.*

Switchgrass (*Panicum virgatum* L.) is a possible crop for meeting biofuel and animal feed/feedstock demands (Mitchell and Schmer, 2012) that can thrive in a wide geographic distribution and under diverse edaphic conditions (Hashemi and Sadeghpour, 2013). Full production is estimated to require 16.9 to 21.3 million ha of land area (McLaughlin et al., 2002). At the same time, a large amount of land is under pine production in the U.S., and loblolly pine (*Pinus taeda* L.) is widely used for timber and timber byproducts. Intercropping switchgrass in loblolly pine stands is hypothesized to provide bioenergy feedstock. Replacing the natural understory, which needs control to avoid competing with pine crops, with switchgrass (Foster et al., 2013) could reduce the amount of herbicide needed. Production of switchgrass from intercropping will also likely provide income during a typical 25-year rotation for loblolly pine. The intercropping system may thus improve productivity and profits per hectare of forest land. In order to recommend this novel intercropping practice to forest owners, there is a critical need for information about the water quantity and quality effects of cellulosic biofuel production.

Switchgrass can reduce soil erosion and wind resistance,

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improve soil carbon stocks through soil retention, and reduce nutrient transport to drainage water (Hashemi and Sadeghpour, 2013). Cultural activities affecting water quality in a loblolly pine/switchgrass forest compared to a typically managed pine forest are (1) the site preparation activities needed to establish switchgrass, (2) annual or bi-annual mowing and baling, and (3) changes in herbicide and fertilizer prescriptions. Herbicide application in a loblolly pine/switchgrass forest ends after the first two years of switchgrass growth. Herbicides are also applied for two years after pine planting to reduce competition for nutrients and space between the pine trees and natural understory in a traditional pine forest. Normally, greater amounts of herbicide are needed for a natural loblolly pine/understory forest because the entire biomass must be sprayed in the pine rows. Additional site preparation can disturb the soil, changing its properties and extending the amount of time with uncovered bare soil; however, once established, switchgrass plants provide dense coverage. Annual harvesting of switchgrass removes biomass from the system, and switchgrass plants can sprout from the root stocks (Hashemi and Sadeghpour, 2013).

Minick et al. (2014) reported that soil NH_4-N and NO_3-N concentrations decreased significantly a year after switchgrass establishment in a plot-scale study in Lenoir County, North Carolina. Albaugh et al. (2014) reported increased transpiration of switchgrass during the growing season and greater total evapotranspiration for loblolly pine than for switchgrass in a loblolly pine/switchgrass plot in Lenoir County, North Carolina. Cacho et al. (2015) reported reduction in soil pore size for a loblolly pine/switchgrass plot in Lenoir County, North Carolina, which resulted in increased ability of the soil to retain water and increased plant water uptake. An earlier report on watershed-scale studies (Muwamba et al., 2015) did not address the potential changes in soil and water nutrients due to the growth of switchgrass, as switchgrass was not fully established in a loblolly pine/switchgrass forest at the time of reporting. We hypothesize that intercropping loblolly pine and switchgrass will reduce nutrient export to drainage water compared to a managed loblolly pine forest or pure switchgrass.

A paired watershed design approach, employed in earlier companion studies (Muwamba et al., 2015; Ssegane et al., 2017), was also used for this study. During the calibration period (before clearcut harvesting), vegetation on the control (mid-rotation loblolly pine) and treatment sites (pure switchgrass and switchgrass intercropped with loblolly pine) had reached canopy closure with similar hydrology, and sites with similar area, slope, drainage design, and soil type were monitored at the same time for developing pretreatment calibration regression relationships. Two watersheds were clearcut harvested, followed by planting in loblolly pine/switchgrass or switchgrass only. The control (mid-rotation loblolly pine) remained unharvested. The objectives of this study were to: (1) quantify the concentrations and loads of nitrogen (N) and phosphorus (phosphate) in drainage water associated with establishment and growth of the switchgrass treatments and compare them with those from a mid-rotation pine forest (control), and (2) compute the treatment effects on drainage water N and phosphate and compare the effects between treatments, i.e., switchgrass intercropped into young loblolly pine (IC) and switchgrass only (SG).

Materials and Methods

Site Location and Layout of Watersheds

The study watersheds are located on Weyerhaeuser land in Carteret County, North Carolina (34.8° N, -76.7° W). Watershed MP (25.9 ha) is mid-rotation thinned loblolly pine with a natural understory (control), watershed IC (26.31 ha) is a loblolly pine/switchgrass forest treatment, and watershed SG (27.1 ha) is a fully switchgrass treatment. The study site is located on a flat coastal plain with a 0.1% gradient and 3 m elevation above sea level (McCarthy et al., 1991). The site's soil type is classified as Deloss fine sandy loam (fine-loamy, mixed, thermic Typic Umbraquult), with pH of about 4 (Beltran et al., 2010). Each watershed is drained by four parallel ditches spaced at 100 m and 1.4 to 1.8 m deep (fig. 1). The watersheds are separated by artificial divides at the midpoint between two parallel ditches. The raised beds (0.30 m) typical for planting loblolly pine trees minimize surface runoff toward the watershed outlet. The surroundings of the site are agricultural land to the east, and the rest is forest. Stand characteristics, drainage design, soil type and properties, and weather parameters for the study site were described elsewhere (Amatya et al., 1998; Amatya and Skaggs, 2011).

Management of Treatment and Control Stands

In 2007, loblolly pine trees were 33 years old on IC and SG, and 10 years old on MP. The trees on IC were harvested from 5 January to 1 April 2009. Shearing and bedding on IC were performed between 13 June and 30 July 2009 using a V-shear blade mounted on the front of a crawler tractor and a special harrow pulled behind a crawler tractor. Shearing and bedding were done to improve growth conditions for pine seedlings in the beds and for switchgrass seeds between the beds. Seedlings were planted by hand on IC at a density of 1087 trees ha^{-1} during 18-20 January 2010 in beds 6.1 m apart. A second V-shear operation was performed in the area between beds on 16 December 2010 to enhance the soil contact of switchgrass seeds.

Tree harvest at SG occurred over a longer period (19 Oct. 2009 to 10 May 2010) due to very wet conditions that hindered trafficability from November 2009 to April 2010. Shearing and raking were implemented over the entire SG area from 12 to 19 April 2011, and raking to windrows was implemented from 20 April to 2 May 2011. Broadleaf weed control using 2-4-D was implemented on 1 August 2011 to prepare IC and SG for planting switchgrass seeds, which occurred on 15 August 2011 using a planter (Land Pride, Salina, Kans.). Hurricane Irene occurred on 27 August 2011, causing flooded conditions and failure of seed germination. Switchgrass was reseeded on IC and SG in April 2012 after broadleaf weed control within the pine beds in late 2011. The areas covered with switchgrass seeds between pine rows on IC were 3 m wide, with the remaining 3.1 m width between

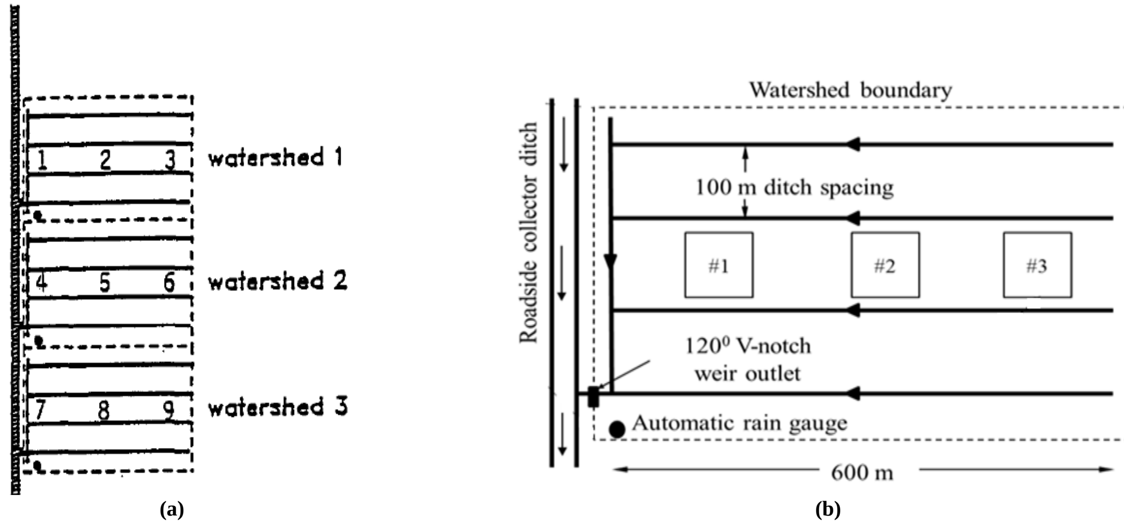

Figure 1. (a) Layout of watersheds: watershed 1 = loblolly pine/switchgrass (IC), watershed 2 = mid-rotation loblolly pine (MP), and watershed 3 = switchgrass only (SG); and (b) monitoring stations for a typical watershed.

the switchgrass edge and pine trees on either side covered by natural understory and weeds. Switchgrass seeds took 30 days to germinate (from field records), with germination percentages of 80% on IC and 65% on SG. The rest of the SG area was covered by weeds.

The pine trees were thinned (from 1087 to 346 trees ha^{-1}) on MP from 18 December 2008 to 9 January 2009 with the aim of improving the stand use efficiency of the available nutrients and water. MP was used as the control because no other operations were performed, and thinning was shown not to have a substantial impact on nutrient cycling (Muwamba et al., 2015). More details on the silvicultural operations for site preparation are given by Muwamba et al. (2015). The pretreatment calibration period was from January 2007 to December 2008, when the pine forests were in mid-rotation growth on MP. The site preparation period for switchgrass establishment was from November 2009, when water quality sample collection restarted, to March 2012. The switchgrass growth period was assumed from April 2012, after second seed broadcast, to April 2014. We used data from April 2012 (second broadcast) to May 2014 (just before fertilizers were applied on all watersheds including the control in June 2014) to represent the switchgrass growth period in this study. We could not use data beyond May 2014 because the paired watershed approach required that the control not be disturbed or fertilized. The typical growing season in the region is from late March to early November (Skaggs et al., 1994).

MEASUREMENT OF RAINFALL AND FLOW RATES

Rainfall was measured with tipping-bucket rain gauges and data loggers (HOBO, Onset Computer Corp., Bourne, Mass.), backed up by standard plastic manual gauges located in open areas near the outlet of each watershed, and processed to obtain daily and annual amounts. Downstream and upstream stages of a 120° V-notch weir at the ditch outlet were measured every 12 min with an automatic recorder (Level TROLL 500, In-Situ Inc., Fort Collins, Colo.) linked with a CR200 data logger (Campbell Scientific, Logan, Utah). Stage data were used to calculate flow rates, which were integrated in daily, monthly, and annual time steps. Possible weir outlet submergence during large storm events was minimized by a pump (installed at the main ditch downstream) equipped with a recorder that was triggered by water level. Details on the hydrologic measurements are given elsewhere (Amatya and Skaggs, 2011; Ssegane et al., 2017).

MONITORING DRAINAGE WATER NITROGEN AND PHOSPHORUS CONCENTRATIONS

An automatic water quality sampler (ISCO-2700, ISCO, Lincoln, Neb.) installed near the 120° V-notch weir outlet collected flow-proportional composite samples. Sampling times were controlled by flow rates calculated from the downstream and upstream stages to collect 150 mL after 200 cm^3 (0.8 mm of watershed area-based water depth) of volume flowed over the V-notch weir. Part of the collected composite volume (500 mL) was transported to the laboratory in ice coolers for total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), and phosphate analysis. Laboratory analytical methods were the ascorbic acid method (Standard Method 4500-P; EPA, 1998) for phosphate, the acid digestion method (Standard Method 4500 Norg B; EPA, 1998) for TKN, the ammonium salicylate method (Standard Method 4500 NH_3G; EPA, 1998) for NH_4-N, and cadmium reduction (Standard Method 4500 NO_3-E; EPA, 1998) for NO_3-N. A Bran Luebbe Autoanalyzer II (Seal Analytical, Mequon, Wisc.) was used for calorimetric analyses of TKN, NH_4-N, and NO_3-N. Detection limits were 0.04 mg L^{-1} for TKN, 0.01 mg L^{-1} for NH_4-N, 0.01 mg L^{-1} for NO_3-N, and 0.01 mg L^{-1} for phosphate. Details on the water quality sampling methods are given elsewhere (Amatya and Skaggs, 2011; Beltran et al., 2010).

DATA ANALYSES

Missing daily flow data for MP (25 Sept. to 20 Oct. 2010) were filled using a regression equation (R^2 = 0.9) with IC flow data. For days when flow volumes were measured with no corresponding sample concentrations, measured concentrations prior to the missing sample times were used to calculate loads. An annual runoff coefficient (ROC) was calculated as the ratio of annual flow to annual rainfall. Loads, calculated as concentration times flow volume for the sample measurement intervals, were recorded as mass per hectare divided by the watershed area. Organic N was calculated by subtracting NH_4-N from TKN, inorganic N was the summation of NH_4-N and NO_3-N, and total N (TN) was the summation of TKN and NO_3-N.

Annual loads and exploratory statistics for nutrient concentrations (mean, minimum, maximum, and standard error) were calculated using Microsoft Excel 2010. Consistent with our earlier study (Muwamba et al., 2015), the paired t-tests for equal samples procedure in Excel was used to test for differences in mean nutrient concentrations and loads at a 5% significance level (α = 0.05). Accordingly, throughout this article, significant differences refer to α = 0.05. A paired watershed approach with bootstrap geometric regression was used to obtain equations for the analyzed nutrients for IC and SG during the calibration, site preparation, and switchgrass growth periods, with control MP acting as a covariate, following the procedure used by Ssegane et al. (2017) for hydrologic variables on these watersheds. Analysis of covariance (ANCOVA) and general linear models (GLM) in SAS (ver. 9.4, SAS Institute, Inc., Cary, N.C.) were used to identify significant differences (α = 0.05) in switchgrass growth and calibration and in switchgrass growth and site preparation regression equations (Beltran et al., 2010; Bishop et al., 2005).

Pretreatment calibration equations for the 2007-2008 data were used with measured data from the treatment period to calculate expected NO_3-N, TKN, and phosphate concentrations and loads had the treatment not been imposed. For example, given a calibration equation (IC = *a*MP + *b*, where *a* and *b* are the significant slope and intercept, respectively), the expected value for IC during the treatment period was calculated using measured MP data for the switchgrass growth period. Treatment effects were calculated as the differences between measured and expected values, with positive and negative values indicating increases and decreases, respectively, in nutrient levels (Beltran et al., 2010; Muwamba et al., 2015). Standard t-tests were used to test the significance of differences between the means (with standard errors) of nutrient effects for a treatment pair. Cumulative load effects were plotted for the site preparation period (Nov. 2009 to Mar. 2012) and switchgrass growth period (Apr. 2012 to Apr. 2014, just before fertilization was applied on all watersheds). Nutrient load effects for the switchgrass growth period was obtained by subtracting the nutrient load effect for the site preparation period from the total cumulative load effect.

RESULTS

CHANGES IN N AND P CONCENTRATIONS DURING SWITCHGRASS GROWTH PERIOD

Table 1 shows the annual rainfall, outflow, and ROC for the three watersheds during the calibration, site preparation, and switchgrass growth periods and for individual years during switchgrass growth. Figures 2 through 5 show the measured TKN, NH_4-N, NO_3-N, and phosphate concentrations, respectively, for the entire study period. The TKN, NH_4-N, NO_3-N, and phosphate concentrations measured during switchgrass growth varied with variations in rainfall and outflow (figs. 2 through 5). Figure 6 shows the changes in measured nutrient concentrations (using NO_3-N as an example) with changes in the hydrograph. The periods in figures 2 through 6 when there were no measured concentrations indicate periods of no flow or very little flow when no samples were taken.

Table 1. Total rainfall, outflow, and average runoff coefficient (ROC) during the calibration (2007 and 2008), site preparation (Nov. 2009 to Mar. 2012), and switchgrass growth (Apr. 2012 to Apr. 2014) periods and for individual years during switchgrass growth.

Watershed	Period or Year	Rainfall (mm)	Outflow (mm)	ROC
IC	Calibration	2561.0	483.0	0.19
	Site preparation	2704.0	693.1	0.26
	Switchgrass growth	3194.9	736.0	0.23
SG	Calibration	2624.0	474.1	0.18
	Site preparation	2537.6	488.8	0.20
	Switchgrass growth	3003.9	965.5	0.34
MP	Calibration	2763.4	569.0	0.21
	Site preparation	2590.2	698.3	0.27
	Switchgrass growth	3037.5	700.9	0.23
IC	2012	1473.0	222.1	0.15
IC	2013	1721.9	513.9	0.30
SG	2012	1350.5	183.1	0.21
SG	2013	1653.4	781.9	0.47
MP	2012	1398.4	219.5	0.16
MP	2013	1639.1	481.4	0.29

The mean TKN, NH_4-N, NO_3-N, and phosphate concentrations for the different experimental phases are shown in table 2. The mean phosphate concentration in 2012 was significantly greater for IC than for MP (p = 0.03). Although not significant (p > 0.05), the mean NH_4-N, NO_3-N, and TKN concentrations were greater for IC and SG than for MP in 2012. The mean phosphate concentration was greater for IC than for MP in 2012. The mean NH_4-N (p = 0.04) and phosphate (p = 0.01) concentrations were significantly lower for SG than for MP in 2013. The annual mean phosphate concentration was significantly (p = 0.03) greater for IC than for SG in 2012. The mean nitrate nitrogen concentration was significantly (p = 0.04) lower for IC than for SG in 2012 (table 2). In 2013, the mean TKN concentration was significantly (p = 0.01) greater for IC than for MP. The mean phosphate concentration was significantly (p = 0.03) greater for IC than for SG, the mean phosphate concentration was significantly (p = 0.04) lower for SG than for MP, and the mean NO_3-N and TKN concentrations were significantly (p = 0.01) greater for SG than for MP in 2013. During the switchgrass growth period, the mean NO_3-N concentration was significantly (p = 0.02) greater for SG than for IC. The mean TKN and NO_3-N concentrations for IC and TKN for SG during the switchgrass growth period were significantly (p = 0.01) lower than during the site preparation period. The mean phosphate concentrations for IC and SG during the switchgrass growth period were lower than the corresponding values during the site preparation period (table 2).

The slopes for treatment regression relationships relative to the control (MP) for TKN concentrations increased for SG

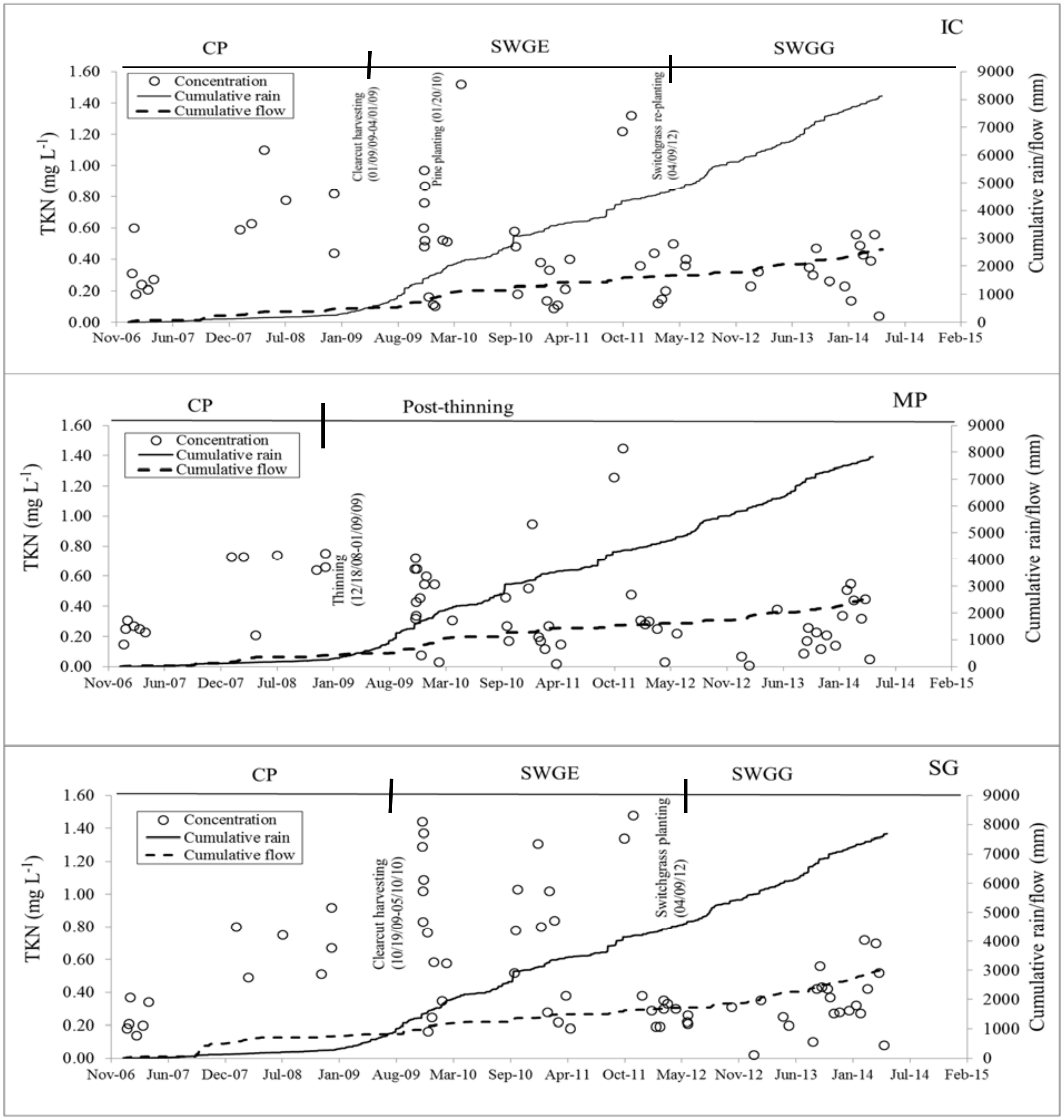

Figure 2. Total Kjeldahl nitrogen (TKN) concentration as a function of time for watersheds IC, MP, and SG during the calibration (CP), site preparation for switchgrass establishment (SWGE), and switchgrass growth (SWGG) periods. For periods without concentrations, there was no flow or the flow volumes were too small to trigger collection of samples by the sampler.

and decreased for IC from calibration to the switchgrass growth period. For the switchgrass growth period, unlike the site preparation period, there were no NO_3-N regression equations because most of the MP values were below the detection limit. The slopes and intercepts for the relationships of IC and SG with MP for phosphate and TKN concentrations during the switchgrass growth period were not significantly different from the site preparation period (table 3).

CHANGES IN N AND P LOADS DURING SWITCHGRASS GROWTH PERIOD

The TKN and NO_3-N loads for IC and SG increased from 2012 to 2013 (table 4). The TKN loads were significantly greater ($p = 0.02$) for SG than for IC in 2012. The phosphate loads increased from 2012 to 2013 on all watersheds (table 4). The TKN and NO_3-N loads for IC and SG during the switchgrass growth period were significantly ($p = 0.04$) lower than during the site preparation period. The TKN and NO_3-N loads were greater for IC and SG than for MP, although not significant in 2012 and 2013. The NH_4-N load was lower for MP than for IC and SG in 2012 (table 4). The phosphate loads in 2013 were greater for IC and SG than for MP (table 4).

The slope of the TKN load relationship for IC relative to MP for the switchgrass growth period was not significantly different from the site preparation period (table 3). The intercept for the TKN load relationships for SG, unlike for IC, for the switchgrass growth period was significantly (p =

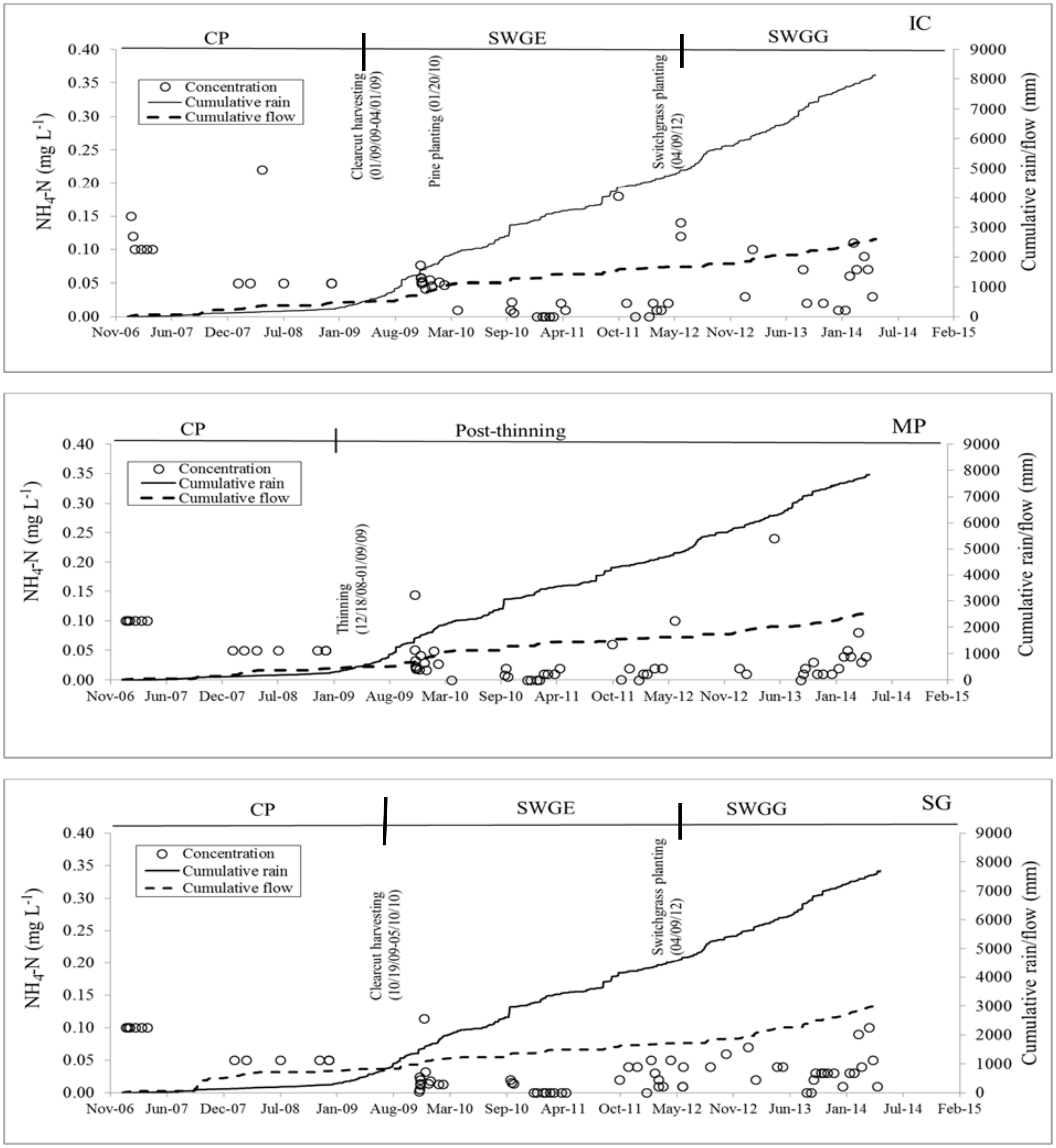

Figure 3. Ammonium nitrogen concentration as a function of time for watersheds IC, MP, and SG during the calibration (CP), site preparation for switchgrass establishment (SWGE), and switchgrass growth (SWGG) periods. For periods without concentrations, there was no flow or the flow volumes were too small to trigger collection of samples by the sampler.

0.02) greater than for site preparation (table 3). For the greatest portion of the switchgrass growth period, the NO_3-N concentrations for MP were below the detection limit, and only a few measurements were available for developing regression equations. Both the slopes and intercepts for the IC-MP and SG-MP phosphate load relationships for the switchgrass growth period were not significantly different ($p = 0.03$) from site preparation after using the GLM procedure described above (table 3).

TREATMENT EFFECTS ON N AND P CONCENTRATIONS AND LOADS DURING SWITCHGRASS GROWTH PERIOD

The calculated average treatment effects on NO_3-N concentration during the switchgrass growth period were -0.12 ±0.04 mg L^{-1} for IC and -0.002 ±0.01 mg L^{-1} for SG (fig. 7). For phosphate, the calculated treatment effects during the switchgrass growth period were -0.02 mg L^{-1} for IC and -0.004 mg L^{-1} for SG. There was a significant ($p = 0.04$) decrease in NO_3-N and phosphate concentrations due to pine/switchgrass treatment compared to MP. The average treatment effects on TKN concentration during the switchgrass growth period were 0.18 ±0.03 mg L^{-1} for IC and

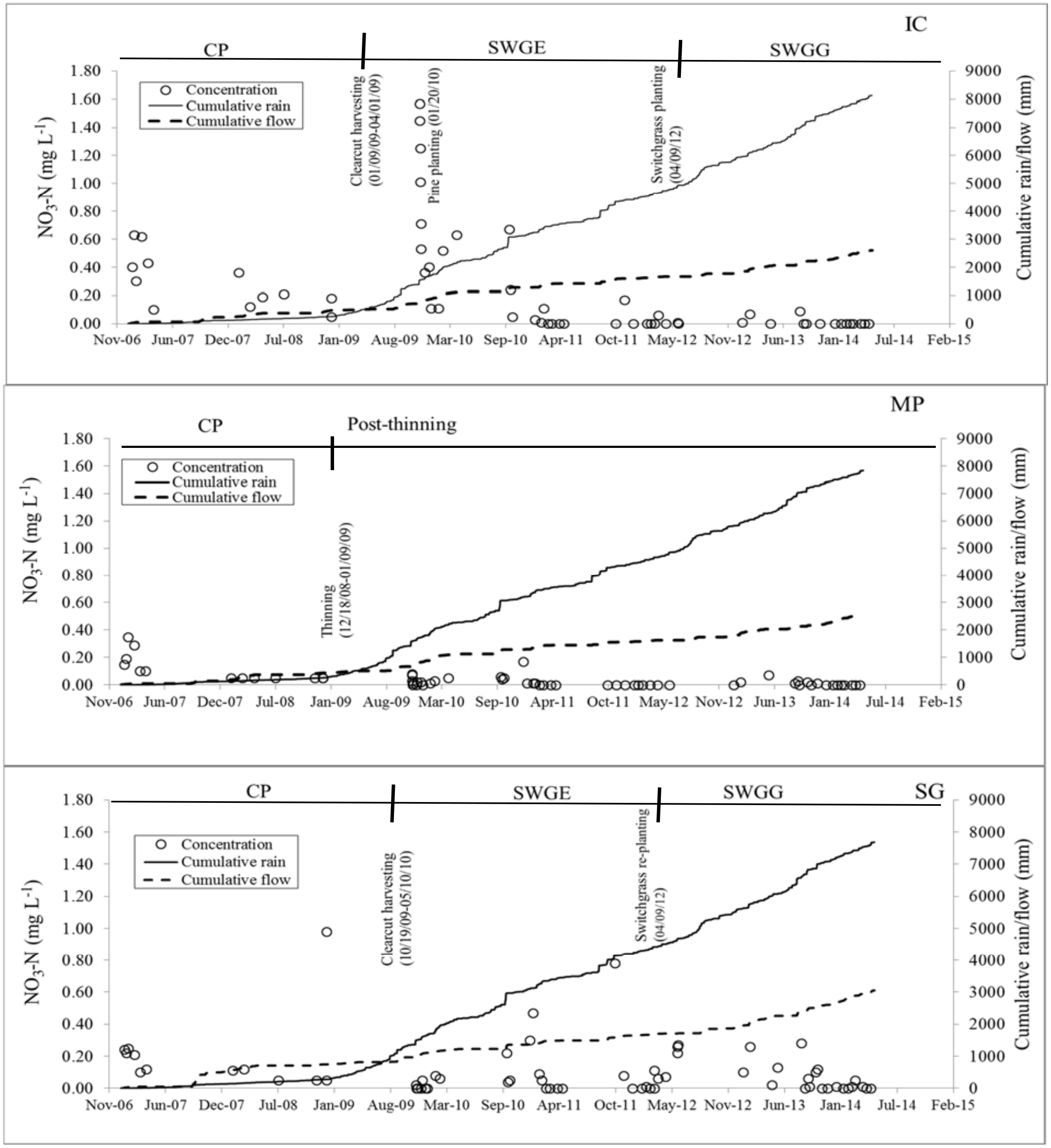

Figure 4. Nitrate nitrogen concentration as a function of time for watersheds IC, MP, and SG during the calibration (CP), site preparation for switchgrass establishment (SWGE), and switchgrass growth (SWGG) periods. For periods without concentrations, there was no flow or the flow volumes were too small to trigger collection of samples by the sampler.

0.13 ±0.02 mg L^{-1} for SG. The NO_3-N concentration treatment effects during the switchgrass growth period were significantly ($p = 0.03$) greater for SG than for IC. The average NO_3-N concentration treatment effect for IC during switchgrass growth (-0.12 ±0.04 mg L^{-1}) was lower with decreased nutrient levels than the treatment effect (0.19 ±0.06 mg L^{-1}) during site preparation (fig. 7).

Total TKN load effects during the switchgrass growth period were 0.92 kg ha^{-1} for IC and 1.69 kg ha^{-1} for SG. Total NO_3-N load effects during the switchgrass growth period were 0.23 kg ha^{-1} for IC and 0.75 kg ha^{-1} for SG (fig. 8). Total phosphate load effects during the switchgrass growth period were -0.43 kg ha^{-1} for IC and -0.70 kg ha^{-1} for SG (fig. 9). The mean NO_3-N load effects during the switchgrass growth period were significantly greater ($p = 0.03$) for SG (0.04 ±0.03 kg ha^{-1}) with increased nutrient levels than for IC (0.02 ±0.01 kg ha^{-1}). However, the phosphate load effects for both IC and SG significantly ($p = 0.01$) decreased for the switchgrass growth period compared to MP (fig. 9). The mean NO_3-N load effects for SG during the switchgrass growth period were significantly ($p = 0.01$) greater than during site preparation. The mean NO_3-N load effects for IC during the switchgrass growth period were significantly ($p = 0.04$) lower with reduced nutrient levels than during site preparation.

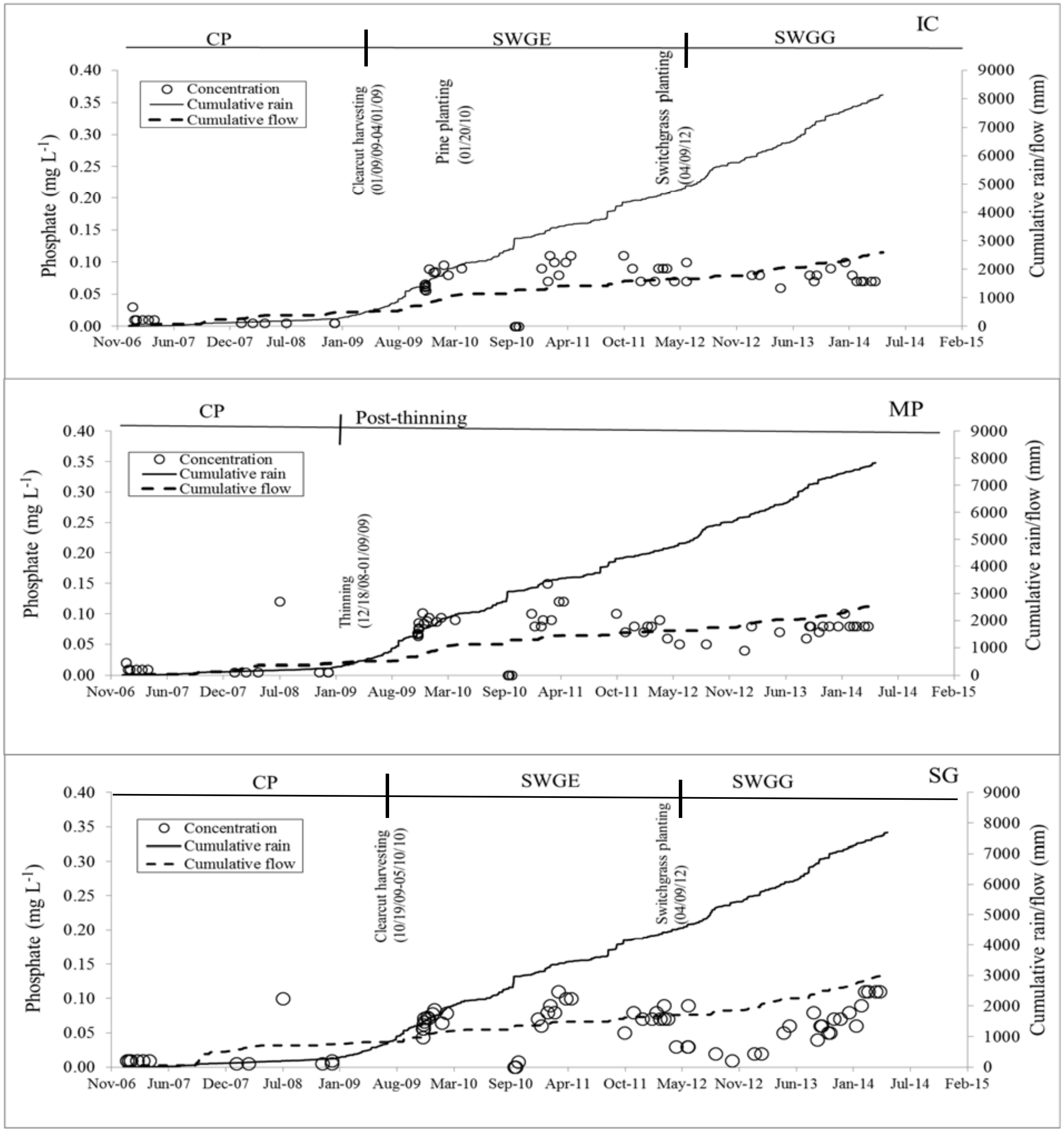

Figure 5. Phosphate concentration as a function of time for watersheds IC, MP, and SG during the calibration (CP), site preparation for switchgrass establishment (SWGE), and switchgrass growth (SWGG) periods. For periods without concentrations, there was no flow or the flow volumes were too small to trigger collection of samples by the sampler.

DISCUSSION

The TKN, NH_4-N, and NO_3-N concentrations for IC and SG increased after long dry periods (figs. 2 through 6), likely due to first-flush effects (Amatya et al., 1998; Beltran et al., 2010; David et al., 2003) associated with large rainfall events. For example, a total of 99.3 mm was received on 7-8 February 2013 and a total of 41.3 mm was received on 13-15 August 2013 on IC. Greater numbers of measurements were made when high amounts of rain were received, e.g., between September 2013 and April 2014 (figs. 2 through 6). The regression slopes for TKN concentrations on IC did not significantly change with respect to MP from site preparation to the switchgrass growth period (table 3). This was probably due to the preference for inorganic N by young pine trees on IC without a significant change in TKN slopes, as documented by Wei et al. (2015). The decreasing treatment effects on NO_3-N from site preparation to the switchgrass growth period for IC (implying an improvement in water quality) were attributed to increasing uptake by both pine and switchgrass over time. Our watershed-scale results are consistent with previous studies. For example, Li et al. (2003) reported that soil nutrient concentrations decreased with increased pine development. Richter et al. (2000) reported that increases in forest stand age decreased soil N due to increasing tree N demand and N accumulation in the tree biomass. Most importantly, in a companion plot-scale study

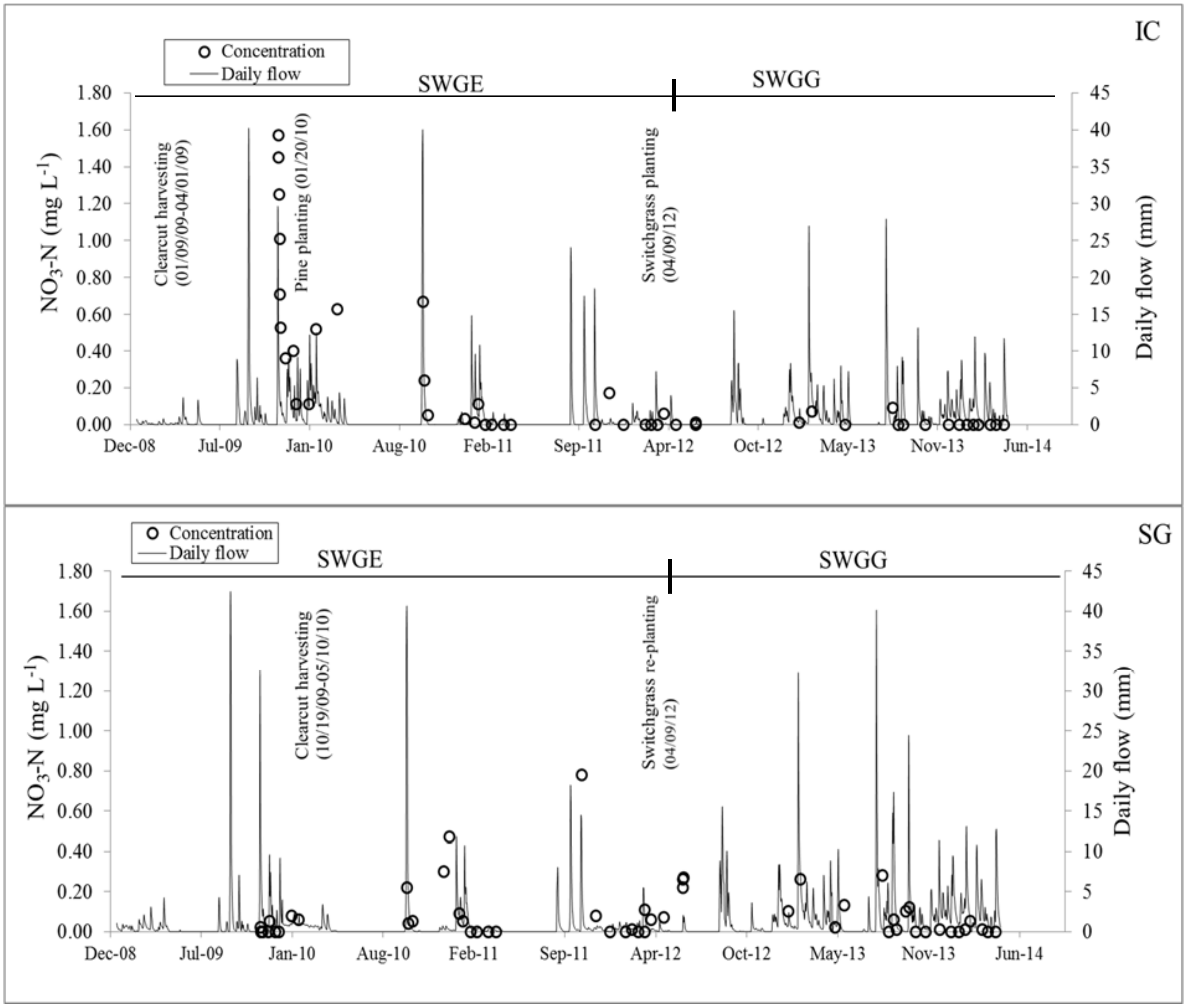

Figure 6. Nitrate nitrogen concentration as a function of time and daily flow (hydrograph) for watersheds IC and SG during the site preparation for switchgrass establishment (SWGE) and switchgrass growth (SWGG) periods. For periods without concentrations, there was no flow or the flow volumes were too small to trigger collection of samples by the sampler.

that also involved switchgrass and pine/switchgrass intercropping in Lenoir County, North Carolina, Minick et al. (2014) found significant reductions in soil NH_4-N and NO_3-N concentrations a year after switchgrass establishment. For the same plot-scale study site, Albaugh et al. (2014) documented that pine transpiration increased with stand age. Albaugh et al. (2014) also reported that pine and switchgrass used more water than switchgrass alone at a site in North Carolina due to additional evapotranspiration from the pine canopy. In their ongoing study, Amatya et al. (2016) calculated larger treatment effects on outflow for a pure switchgrass site than for a pine/switchgrass site when compared with the MP control.

The TN concentration trend (SG > IC > MP) in 2012 and 2013 was attributed to differences in uptake, with the highest uptake on MP, followed by IC and then SG, and on the quality of mineralizable materials, as mid-rotation pine has lower-quality materials than young pine. The MP treatment established deep root systems, potentially with larger uptake ability, while IC had both herbaceous switchgrass with dense biomass roots within about 30 cm depth and young woody pine with deeper root systems, and SG had herbaceous grass of greater mineralizable quality. The quality of mineralizable materials is related to their content; for example, nutrient changes in forests have indicated that the lignin and cellulose contents of plant materials increase with stand age, resulting in N immobilization, lower quality of mineralizable materials, and reduced mineralization rates (Gurlevik et al., 2004; Flavel and Murphy., 2006; Muwamba et al., 2015).

The consistent trend in annual mean phosphate concentrations (IC > MP> SG) in 2012 and 2013 was attributed to P fixation by the soil due to low pH (~4 for the study site), with the watershed that recorded the greatest initial P concentration also recording the greatest drainage water concentration in both years. A similar phosphate concentration trend was observed during site preparation (Muwamba et al., 2015), indicating that, for acidic conditions, a site with a greater initial concentration will continue to record a greater concentration over time. Relationships between phosphate concentration and soil characteristics (Fe, Al, and pH) were documented in other studies. In those soil reactions, acidic conditions led to fixation of P by the soil due to increased binding of P by soil Fe and Al at low pH, as documented by Srinivasarao et al. (2007). The P concentrations trend (IC >

Table 2. Mean nitrogen and phosphorus concentrations (with ranges in parentheses) during calibration (Jan. 2007 and Dec. 2008), site preparation (Nov. 2009 to Mar. 2012), and switchgrass growth (Apr. 2012 to Apr. 2014) periods and for individual years during switchgrass growth.[a]

Period or Year	Watershed	TKN ($mg\ L^{-1}$)	NH_4-N ($mg\ L^{-1}$)	NO_3-N ($mg\ L^{-1}$)	PO_4^{3-} ($mg\ L^{-1}$)
Calibration	IC	0.51 (0.18-1.10)	0.10 (0.05-0.22)	0.30 (0.05-0.63)	0.01 (0.01-0.03)
Site preparation	IC	0.48 (0.09-1.52)***	0.04 (0.0-0.18)	0.5 (0.0-1.57)***	0.08 (0.0-0.11)
Switchgrass growth	IC	0.35 (0.04-0.56)	0.06 (0.01-0.14)	0.01 (0.0-0.09)**	0.07 (0.0-0.10)
Calibration	SG	0.47 (0.09-1.52)	0.08 (0.05-0.10)	0.44 (0.05-2.0)	0.02 (0.01-0.10)
Site preparation	SG	0.69 (0.16-1.48)***	0.02 (0.0-0.11)	0.15 (0.0-0.78)	0.07 (0.0-0.11)
Switchgrass growth	SG	0.33 (0.02-0.72)	0.04 (0.0-0.10)	0.08 (0.0-0.28)	0.06 (0.01-0.11)
Calibration	MP	0.46 (0.15-0.75)	0.07 (0.05-0.10)	0.28 (0.05-2.10)	0.02 (0.01-0.12)
Site preparation	MP	0.43 (0.02-1.45)	0.02 (0.0-0.14)	0.06 (0.0-0.27)	0.09 (0.0-0.15)
Switchgrass growth	MP	0.24 (0.01-0.55)	0.04 (0.0-0.24)	0.01 (0.0-0.07)	0.07 (0.01-0.10)
2012	IC	0.31 (0.12-0.50)	0.05 (0-0.14)	0.02 (0-0.20)	0.08 (0.07-0.1)*
2013	IC	0.31 (0.23-0.47)*	0.04 (0.01-2.0)	0.02 (0.0-0.09)	0.08 (0.07-0.10)
2012	SG	0.33 (0.19-0.97)	0.03 (0-0.06)*	0.10 (0-0.27)*	0.06 (0.01-0.09)*
2013	SG	0.30 (0.02-0.56)	0.03 (0-0.07)	0.08 (0-0.28)	0.05 (0.02-0.08)*
2012	MP	0.23 (0.03-0.31)	0.03 (0-0.10)	0.00 (0.0-0.0)	0.07 (0.05-0.09)
2013	MP	0.17 (0.01-0.38)*	0.04 (0-0.24)*	0.02 (0.0-0.07)*	0.07 (0.04-0.08)*

[a] Asterisks (*) indicate statistical significance ($\alpha = 0.05$): IC* indicates that IC is significantly greater or less than MP in a given year, MP* indicates that MP is significantly greater or less than SG in a given year, SG* indicates that SG is significantly greater or less than IC in a given year, IC** indicates that IC is significantly greater or less than SG for a given experimental phase, and IC*** or SG*** indicate that the value for site preparation is significantly greater or less than the value for switchgrass growth. The p-values are provided in the text.

Table 3. Bootstrap geometric mean regression relationships between switchgrass/pine watershed (IC) and control (MP) and between pure switchgrass watershed (SG) and control (MP). Regression equations are shown for nutrient concentrations and loads for the calibration (Jan. 2007 and Dec. 2008), site preparation (Nov. 2009 to Mar. 2012), and switchgrass growth (Ap. 2012 to Apr. 2014) periods.[a]

Period	IC-*N*	SG-*N*	Nutrient	IC Equation	SG Equation
Concentration ($mg\ L^{-1}$)					
Calibration	21	20	TKN	IC = 0.92MP + 0.03 (R^2 = 0.68)	SG = 0.85 MP + 0.01 (R^2 = 0.74)
Site preparation	34	36	TKN	IC = 0.92MP + 0.04 (R^2 = 0.71)	SG = 0.95MP + 0.13 (R^2 = 0.75)
Switchgrass growth	14	17	TKN	IC = 0.83MP + 0.14 (R^2 = 0.63)	SG = 1.19MP + 0.10 (R^2 = 0.42)
Calibration	22	21	NO_3-N	IC = 1.80MP + 0.12 (R^2 = 0.56)	SG = 0.69MP + 0.05 (R^2 = 0.75)
Site preparation	36	34	NO_3-N	IC = 11.87MP + 0.06 (R^2 = 0.83)	SG = 2.64MP + 0.03 (R^2 = 0.70)
Switchgrass growth	-	-	NO_3-N	NA	NA
Calibration	23	23	PO_4^{3-}	IC = 1.41MP – 0.002 (R^2 = 0.95)	SG = 0.95MP (R^2 = 0.94)
Site preparation	39	36	PO_4^{3-}	IC = 0.85MP + 0.01 (R^2 = 0.87)	SG = 0.84MP – 0.002 (R^2 = 0.77)
Switchgrass growth	17	19	PO_4^{3-}	IC = 1.04MP + 0.001 (R^2 = 0.39)	SG = 1.76MP – 0.06 (R^2 = 0.55)
Load ($kg\ ha^{-1}$)					
Calibration	23	23	TKN	IC = 0.95MP + 0.03 (R^2 = 0.45)	SG = 2.08MP – 0.08 (R^2 = 0.54)
Site preparation	37	37	TKN	IC = 1.06MP + 0.01 (R^2 = 0.66)	SG = 1.21MP – 0.01 (R^2 = 0.73)
Switchgrass growth	13	16	TKN	IC = 1.14MP + 0.02 (R^2 = 0.69)	SG = 1.39MP + 0.03 (R^2 = 0.59)
Calibration	22	22	NO_3-N	IC = 5.73MP – 0.02 (R^2 = 0.43)	SG = 5.08MP – 0.02 (R^2 = 0.71)
Site preparation	39	36	NO_3-N	IC = 7.51MP + 0.02 (R^2 = 0.83)	SG = 2.31MP + 0.001 (R^2 = 0.86)
Switchgrass growth	-	-	NO_3-N	NA	NA
Calibration	23	22	PO_4^{3-}	IC = 2.52MP – 0.001 (R^2 = 0.55)	SG = 2.88MP – 0.001 (R^2 = 0.68)
Site preparation	38	37	PO_4^{3-}	IC = 0.81MP + 0.001 (R^2 = 0.84)	SG = 0.65MP (R^2 = 0.86)
Switchgrass growth	18	15	PO_4^{3-}	IC = 1.13MP (R^2 = 0.69)	SG = 1.28MP – 0.01 (R^2 = 0.73)

[a] IC-*N* and SG-*N* are the number of data pairs used for the IC and SG regression equations with MP, respectively. NA indicates no equation because most MP concentrations were below detection limits during the switchgrass growth period. Slopes and intercepts of IC-MP and SG-MP relationships for phosphate and TKN concentrations and loads during switchgrass growth were not significantly different from site preparation. The p-values are provided in the text.

MP > SG) has been observed since the beginning of the experiment, regardless of the management of the sites, because P was fixed at all the sites.

The differences in TKN and NO_3-N loads for IC and MP in 2012 and 2013 were attributed to differences in concentrations and outflows, with IC having greater outflow than MP (tables 2 and 4). The differences in TKN and NO_3-N loads for SG and MP in 2012 and 2013 were also due to differences in concentrations and outflows, with SG having greater concentrations in 2012 and greater outflows in 2013 than MP (tables 2 and 4). The differences in TKN and NO_3-N loads for IC and SG in 2012 and 2013 were probably due to lower uptake by SG than by IC in 2012 and greater outflow on SG in 2013. Nitrate-N load effects significantly increased during the switchgrass growth period, likely due to greater outflow than during site preparation. The NO_3-N load effects during the switchgrass growth period were greater for SG than for IC, also due to greater outflow in 2013, as shown in table 4. Cumulative rainfall and outflow are shown in figures 2 through 5. Greater rainfall intensity was reported to increase nutrient transport by Lee et al. (2000). For example, in 2013, as a result of heavy rainfall (295.1 mm) in July, SG yielded the largest NO_3-N (0.27 kg ha^{-1}) and phosphate (0.076 kg ha^{-1}) loads for that month (fig. 4 and table 4). In August 2012, very large amounts of rainfall (302.3 mm) and outflow (88.4 mm) were recorded on SG, and very high TKN (0.86 kg ha^{-1}) and NO_3-N (0.09 kg ha^{-1}) loads were calculated (fig. 2 and table 4). In February 2013, high outflow (112.3 mm) on IC yielded high exports of NH_4-N (0.10 kg ha^{-1}), TKN (0.37 kg ha^{-1}), NO_3-N (0.09 kg ha^{-1}), and phosphate (0.08 kg ha^{-1}) (figs. 2 through 5;

Table 4. Nutrient loads per unit area for the study watersheds during the calibration (Jan. 2007 and Dec. 2008), site preparation (Nov. 2009 to Mar. 2012), and switchgrass growth (Apr. 2012 to Apr. 2014) periods and for individual years during switchgrass growth.[a]

Period or Year	Watershed	Flow (mm)	Runoff Coefficient	TKN ($kg\ ha^{-1}$)	NH_4-N ($kg\ ha^{-1}$)	NO_3-N ($kg\ ha^{-1}$)	PO_4^{3-} ($kg\ ha^{-1}$)
Calibration	IC	483.0	0.19	3.17	0.39	0.30	0.03
Site preparation	IC	693.1	0.26	4.86**	0.20	3.18**	0.62
Switchgrass growth	IC	736.0	0.23	3.17	0.46	0.30	0.74
Calibration	SG	474.1	0.18	3.75	0.41	0.89	0.09
Site preparation	SG	488.8	0.20	4.47**	0.25	1.47**	0.33
Switchgrass growth	SG	965.5	0.34	3.44	0.50	0.89	0.53
Calibration	MP	569.0	0.21	2.42	0.24	0.14	0.03
Site preparation	MP	698.3	0.27	3.25	0.20	0.29	0.66
Switchgrass growth	MP	700.9	0.23	2.10	0.56	0.14	0.61
2012	IC	222.1	0.15	0.59*	0.06	0.03	0.18
2013	IC	513.9	0.30	1.62	0.27	0.26	0.40
2012	SG	183.1	0.21	1.44	0.12	0.25	0.08
2013	SG	781.9	0.47	2.18	0.19	0.80	0.43
2012	MP	219.5	0.16	0.27	0.04	0.00	0.12
2013	MP	481.4	0.29	0.96	0.40	0.14	0.35

[a] IC* indicates that the load value for IC is significantly greater or less than the value for SG in a given year; IC** or SG** indicate that the value during site preparation is significantly greater or less than the value during switchgrass growth. The p-values are provided in the text.

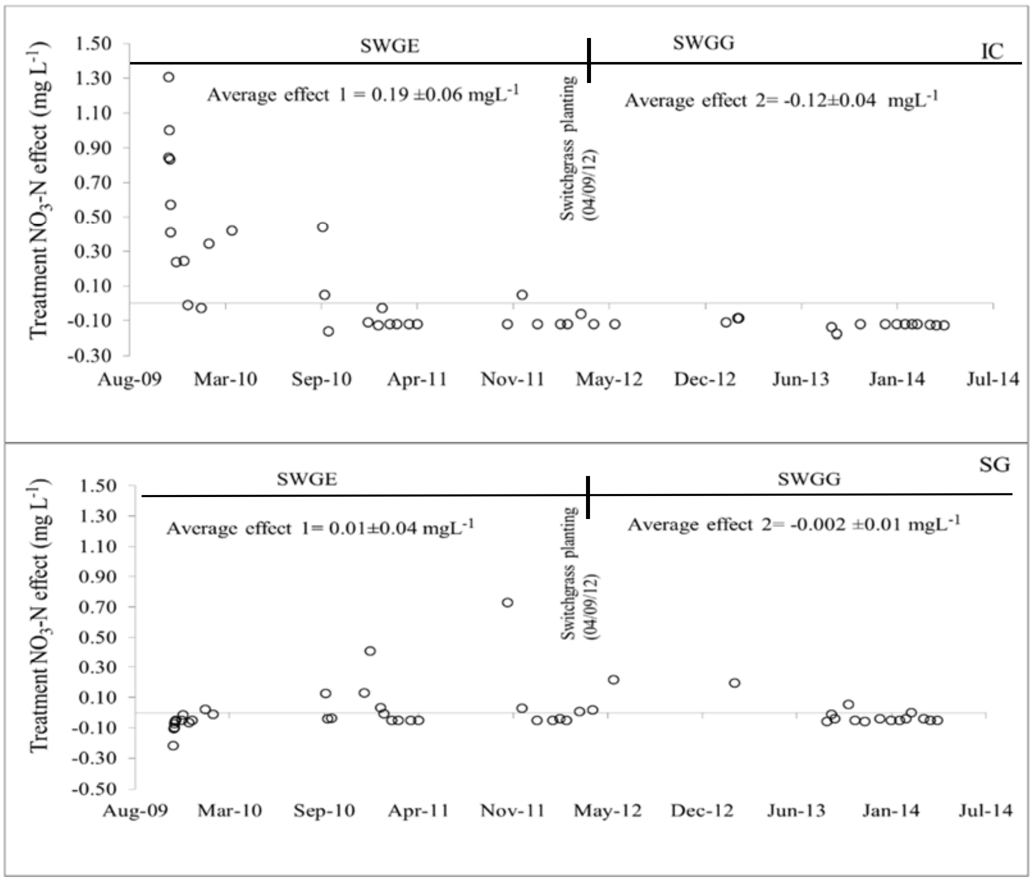

Figure 7. Treatment effects for nitrate nitrogen (NO_3-N) concentration as a function of time for IC and SG during site preparation (average effect 1, SWGE) and switchgrass growth (average effect 2, SWGG). The treatment effect was different between the measured concentration and the mean concentration calculated by the regression relationship for the 2007-2008 calibration period between the treatment (IC or SG) and control (MP). Treatment effects for site preparation are from Muwamba et al. (2015). The significance tests with p-values are provided in the text.

table 4). Similarly, in August 2012 on IC, with large amounts of rainfall (321.4 mm) and outflow (61.7 mm), large loads of NH_4-N (0.02 $kg\ ha^{-1}$), TKN (0.14 $kg\ ha^{-1}$), and phosphate (0.05 $kg\ ha^{-1}$) were exported (fig. 2, 3, and 5; table 4).

The phosphate load trends (IC > MP > SG in 2012 and SG > IC > MP in 2013) were dictated by differences in both concentration and outflow (table 4). Increased outflow might have led to increased P desorption. For example, the following phosphate trends were identified: (1) IC had greater loads than MP in 2012 and 2013 due to greater concentrations and outflows, (2) SG had lower loads than MP in 2012 due to lower concentrations and outflows, (3) SG had greater loads than MP in 2013 due to greater outflows, (4) IC had greater loads than SG in 2012 due to greater concentrations

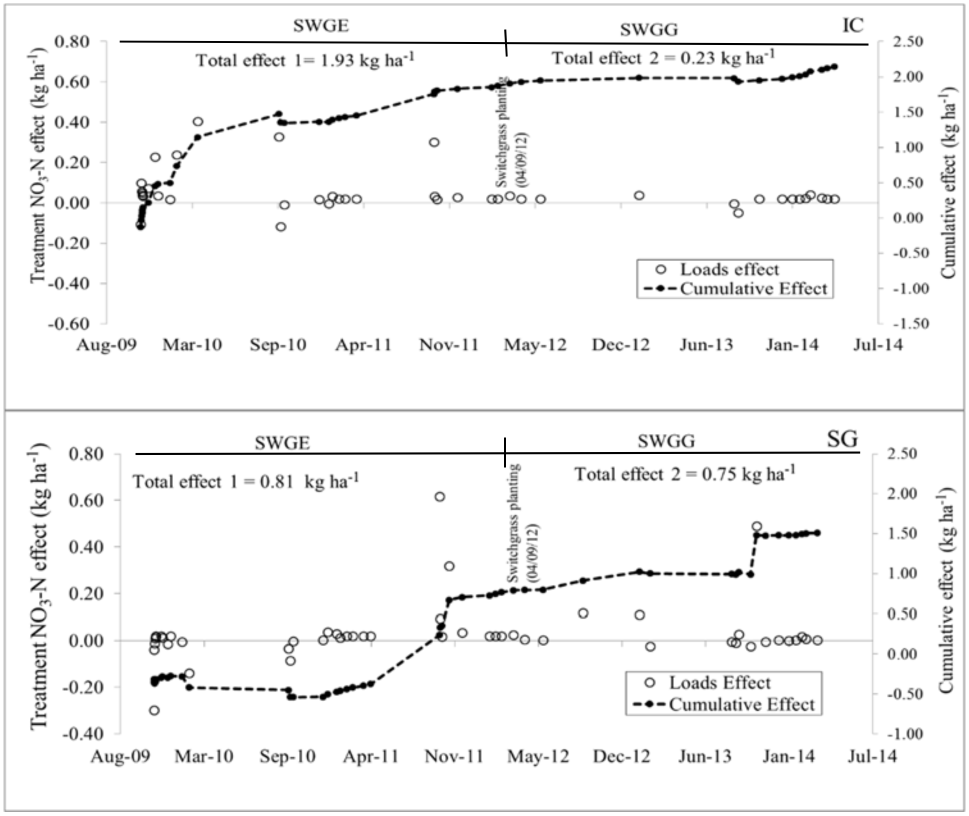

Figure 8. Treatment effects for nitrate nitrogen (NO_3-N) as a function of time for IC and SG for site preparation (total effect 1, SWGE) and switchgrass growth (total effect 2, SWGG). The treatment effect was different between the measured concentration and the mean concentration calculated by the regression relationship for the 2007-2008 calibration period between treatment (IC or SG) and control (MP). Treatment effects for site preparation are from Muwamba et al. (2015). The significance tests with p-values are provided in the text.

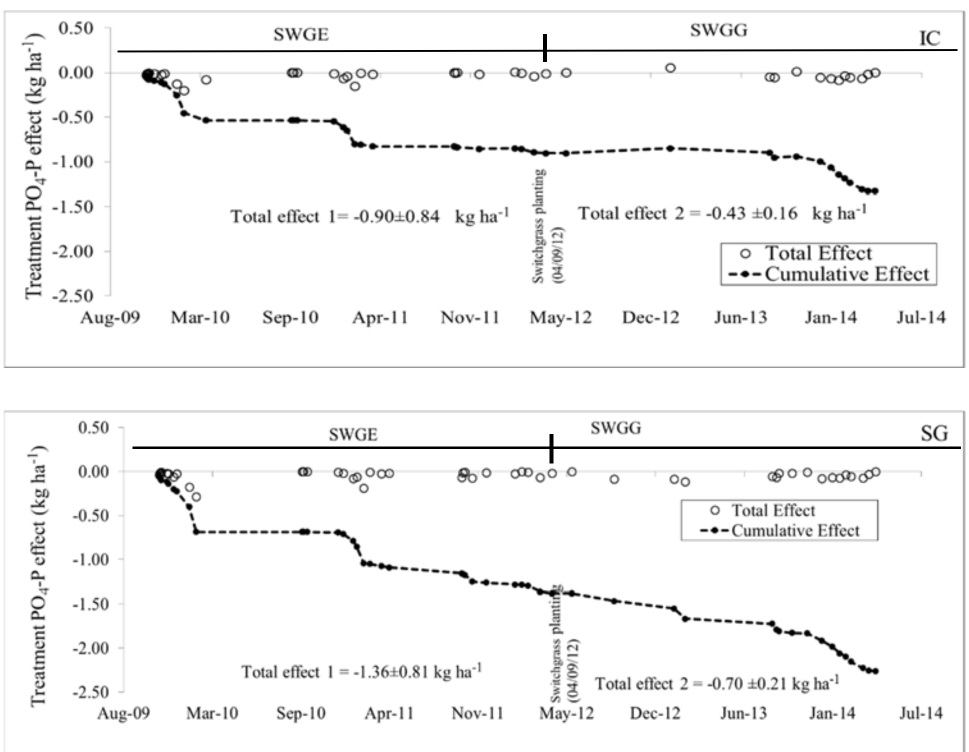

Figure 9. Treatment effects for phosphate (PO_4-P) loads as a function of time for IC and SG for site preparation (total effect 1, SWGE) and switchgrass growth (total effect 2, SWGG). The treatment effect was different between the measured concentration and the mean concentration calculated by the regression relationship for the 2007-2008 calibration period between treatment (IC or SG) and control (MP). Treatment effects for site preparation are from Muwamba et al. (2015). The significance tests with p-values are provided in the text.

and outflows, and (5) SG had greater loads than IC in 2013 due to greater outflows. Phosphorus dynamics in watershed-scale studies have been linked to flow volumes in other studies. McDowell et al. (2001) reported that P desorption increased with the volume and residence time of water flowing over sediment. Gburek et al. (2000), studying P management at watershed scale, also reported that increased flow volume increased the transport of sediment-bound P downstream due to increased detachment of soil.

CONCLUSION

The TKN, NO_3-N, and phosphate concentrations for the pine/switchgrass forest decreased during the switchgrass growth period. The improvement in water quality for the pine/switchgrass forest in 2012-2014 was reflected in reductions of N and P concentrations compared to the traditional mid-rotation pine forest (control). The results also showed that TKN and NO_3-N concentrations and loads for the pine/switchgrass forest during the switchgrass growth period were significantly lower than the earlier values during the site preparation period. Nitrate N loads were greater for the pure switchgrass site than for the pine/switchgrass forest during switchgrass growth, possibly due to greater outflow. Switchgrass-pine intercropping may be a valuable method of producing a sustainable bioenergy crop on pine forest lands while improving water quality.

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