

Environmental Effects over the First 2½ Rotation Periods of a Fertilised Poplar Short Rotation Coppice

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Abstract

A short rotation coppice (SRC) with poplar was established in a randomised fertilisation experiment on sandy loam soil in Potsdam (Northeast Germany). The main objective of this study was to assess if negative environmental effects as nitrogen leaching and greenhouse gas emissions are enhanced by mineral nitrogen (N) fertiliser applied to poplar at rates of 0, 50 and 75 kg N ha⁻¹ year⁻¹ and how these effects are influenced by tree age with increasing number of rotation periods and cycles of organic matter decomposition and tree growth after each harvesting event. Between 2008 and 2012, the leaching of nitrate (NO₃⁻) was monitored with self-integrating accumulators over 6-month periods and the emissions of the greenhouse gases (GHG) nitrous oxide (N₂O) and carbon dioxide (CO₂) were determined in closed gas chambers. During the first 4 years of the poplar SRC, most nitrogen was lost through NO₃⁻ leaching from the main root zone; however, there was no significant relationship to the rate of N fertilisation. On average, 5.8 kg N ha⁻¹ year⁻¹ (13.0 kg CO_{2equ}) was leached from the root zone. Nitrogen leaching rates decreased in the course of the 4-year study parallel to an increase of the fine root biomass and the degree of mycorrhization. In contrast to N leaching, the loss of nitrogen by N₂O emissions from the soil was very low with an average of 0.61 kg N ha⁻¹ year⁻¹ (182 kg CO_{2equ}) and were also not affected by N fertilisation over the whole study period. Real CO₂ emissions from the poplar soil were two orders of magnitude higher ranging between 15,122 and 19,091 kg CO₂ ha⁻¹ year⁻¹ and followed the rotation period with enhanced emission rates in the years of harvest. As key-factors for NO₃⁻ leaching and N₂O emissions, the time after planting and after harvest and the rotation period have been identified by a mixed effects model.

Keywords Nitrogen · Populus spp. · Fertilisation · Greenhouse gas emission · Leaching

Introduction

The global climate is affected by an increase of CO₂ concentration in the atmosphere [1]. According to the Paris climate

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summit in December 2015, international consensus exists on the need to limit the global increase in temperature to 1.5-2.0 °C and to achieve climate neutrality in the second half of this century [2]. One suitable strategy for mitigating the accumulation of CO₂ in the atmosphere may be the production and use of energy from biomass produced in silvicultural and agricultural sites [3, 4] in combination with the build-up of stable carbon pools in the soil [5, 6]. Most promising biomass yields are expected from wood production via short rotation coppices (SRC) on nutrient-enriched arable land [7]. But also under unfavourable site conditions as in Lusatian reclaimed mining sites of Eastern Germany, fast-growing trees have been recommended as a promising element of agroforestry, which is considered as an appropriate system for the production of fuelwood in rural areas [8]. Fertilisation can increase the yield of woody biomass and thus its fossil fuel substitution potential [9, 10]. However, it must be taken into account that due to intensive soil management, environmental impacts such as amplified NO₃⁻ leaching and greater emission of



GHG from the soil may occur during the production of trees [11–13]. Alongside tillage and harvesting, N fertilisation has often been identified as the operation responsible for NO₃ leaching and the emission of N₂O [14, 15]. Balasus et al. [16] had studied the environmental effects of N fertilisation (75 kg N) in a new established SRC on sandy loam soil in Potsdam (Northeast Germany). After the first rotation period, they found fertiliser-induced leaching rates of 40 kg N ha⁻¹ year⁻¹ in the poplar SRC. Also fertigation, which might be an option to increase biomass yield, led to considerably increased environmental impacts, which could not be compensated by an increase of biomass yields [17]. During the life cycle of an SRC poplar plantation, the initial stage has a risk for increased GHG emissions, resulting from enhanced mineralisation of accumulated soil organic matter, particularly if agricultural crops were produced during the preceding period [16, 18]. Whether or not a new bioenergy crop is beneficial or worse related to the former land use (arable, pasture or forest/semi-natural) had been studied earlier for example by Hillier et al. [19] for different bioenergy crops in England and Wales. Their life-cycle assessment (LCA) showed that among the studied crops, SRC with poplar had a mostly beneficial impact in reducing GHG emissions by offsetting emissions from management under the previous land use, and considering fossil fuel C displaced. Another LCA of net GHG fluxes for different bioenergy cropping systems in Pennsylvania (USA) revealed that besides switchgrass, hybrid poplar provided the largest net GHG sink of above 400 g CO_{2equ}-C m⁻² year⁻¹ for biomass gasification for electricity generation [20]. A Mediterranean study also substantiated the suitability of a poplar bioenergy system in terms of an energy balance and environmental perspective at least as long as water supply is not limited [21].

A further important aspect affecting the assessment of an SRC is the uprooting and its transformation to arable land, which may result in increased mineralisation of root residues and CO₂ production [22]. The longevity of SRC of 20 and more years, however, explains why this final phase of reconversion has not been fully assessed in terms of environmental pollution. Also, the harvest of above-ground biomass at the end of each rotation can have an impact on the remaining biomass of roots and on the microbiological activity in the rhizosphere [23]. A decay of roots may be linked with an increase in CO₂ emissions after harvest of the above-ground biomass, but there is only little knowledge on these belowground processes.

In this study, which is a follow-up to Balasus et al. [16], GHG emissions and NO₃⁻ leaching have been measured over multiple harvest rotations in order to fill knowledge gaps and to give new and comprehensive information about the sustainability of SRC. The overall questions are whether the negative environmental effects like N leaching and greenhouse gas emission are enhanced by N fertilisation and how these effects

are related to tree age with increasing number of rotation periods and to cycles of organic matter decomposition and tree growth after each harvesting event. For both, GHG emissions (CO₂, N₂O) and NO₃⁻ leaching, mixed effect models were established in order to clarify the relevance of driving forces for these environmental effects and to support the assessment of biomass production by SRC.

Materials and Methods

Study Site

The study was performed between April 2008 and December 2012 on a new experimental field at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) in Potsdam, Germany (52.437358° N, 13.017322° E) at an altitude of 35 m above sea level. The landscape and the soil of the Potsdam region belong to the top moraine and were formed during the last glacial period 20,000 to 14,000 years ago [24]. According to the World Reference Base for Soil Resources (WRB), the soil can be classified as a Gleyic Cambisol, which is representative for the Northeastern region of Germany. According to the German Weather Service (DWD), in the long term between the years 1995 and 2009, the mean air temperature was 9.6 °C (±0.7 °C) and the mean annual precipitation rate was 599 mm (±130 mm).

Experimental Design

Biological experiments such as the growth of energy crops contain numerous sources of variability, which have to be taken into account. This is particularly true for perennial SRC with a larger scale and a longer duration of experimental plots compared to annual crops [25]. In order to minimise the spatial heterogeneity of the soil, a soil conductivity map was prepared for the experimental field (EM-38 electromagnetic soil conductivity meter). Thereby, homogeneous areas reflecting largely homogenous soil texture could be identified. By this way, uniform repetition blocks within the experimental area were selected. Before plot installation, annual cereals had been grown on the field with no-till management. The experimental field was established in April 2008 after an annual crop of winter rye had been ploughed in into a soil depth of 20 cm. Cuttings of poplar Populus maximovizcii x P. nigra (clone Max 4) were set on 24 and 25 April 2008 with a plant density of 11,000 per hectare. As shown in Fig. 1, a randomised, block design was established in a plot of 1800 m². The whole plot had four blocks that were split into five subplots (9 m × 10 m) resulting in four replicates for the treatment fertilisation. Two subplots per block were not used in this study; for more details see [16].



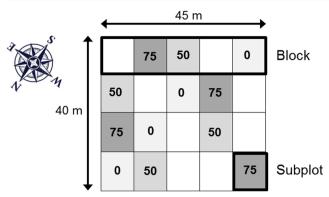


Fig. 1 The whole plot of the SRC with poplar fertilised at three different rates (kg N ha^{-1} year⁻¹)

In another SRC only 300 m away from the SRC under study, it was shown earlier that biomass yield was not affected by N fertilisation at rates higher than 75 kg N ha⁻¹ year⁻¹ [26]. Therefore, in this SRC, the maximum fertilisation rate was set to 75 kg N ha⁻¹ year⁻¹, which is considerably lower compared to studies of Adegbidi et al. [27] and Hellebrand et al. [28] with fertilisation rates of up to 150 kg N ha⁻¹ year⁻¹. Mineral N fertiliser was added manually to the subplots as calcium ammonium nitrate (mixture of NH₄NO₃ and CaCO₃ with a mass N:CaCO₃ ratio of 1:1) at rates of 0, 50 and 75 kg N ha⁻¹ year⁻¹. Sites for gas measurements were fertilised separately with an equivalent and exact amount of calcium ammonium nitrate representing the fertilisation rates. In the first year, the N fertiliser was applied in June; in the other years, from 2009 to 2012 in April (Fig. 2). Only the high fertilisation level was applied in split doses with 50 kg and 25 kg N ha⁻¹ year⁻¹. Poplar stems were harvested manually with a chain saw in December 2009 and December 2011. The system boundary is given by the experimental field down to a soil depth of 0.9 m. The fate of N as NO_3^- leaching in seepage water and N_2O gaseous emissions to the atmosphere are the focus of this study.

Meteorological Data

For the first year (April 2008–May 2009), results for air temperature and rainfall were derived from the DWD in Potsdam. From June 2009 to December 2012, climatic factors were monitored by a weather station (TOSS GmbH Potsdam) located 300 m from the experimental field. Air temperature and precipitation sums were logged every minute. For chemical characterisation of the rainwater and calculation of the airborne N deposition rate, bulk deposition was collected in special glass cylinders (Ø 10.1 cm) and stored in polyethylene bottles. Rainwater samples were preserved frozen for NH₄⁺, NO₂⁻ and NO₃⁻ concentration analysis by flow-injection and spectrometric detection (MLE, Dresden). The analysis was performed at least once a week.

Rhizosphere of Poplar

The study of the poplar rhizosphere was restricted to the fine roots, which are best known for ectomycorrhization [29]. Soil from the subplots was sampled every year in July in 5-cm layers down to a depth of 40 cm according to the *sequential coring-procedure* [30]. Duplicate samples were stored at +4 °C until the mass of fine roots and the amount of mycorrhization were determined. The samples were washed in a sieve with a mesh size of 1 mm in order to separate the roots from soil particles. Only the fine roots up to a diameter of 2 mm were considered for

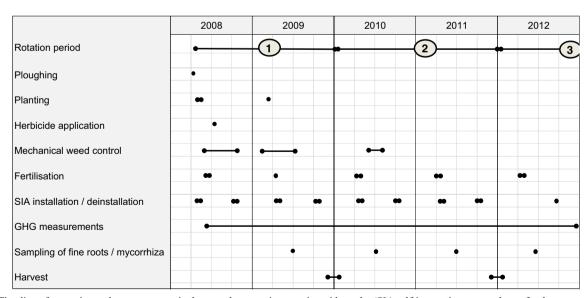


Fig. 2 Timeline of managing and measurements in the new short rotation coppice with poplar (SIA self-integrating accumulators for the measurement of NO_3^- leaching)



further analyses. The dry weight of fine roots was obtained after drying at 85 °C and weighed with a special accuracy weighing machine (XS205, Mettler Toledo, Greifensee, Switzerland) with a precision of ± 0.01 mg. Average values are given for the whole rooted zone between 0 and 40 cm. The N content of fine roots was measured by using a Euro EA - CHNSO Elemental Analyser (HEKAtech GmbH, Wegberg, Germany). The amount of mycorrhization was derived from the fine roots of the upper soil layer 0–10 cm. The estimation was based on five root clusters of each sample, applying the "gridline intersection" method [31].

Soil Characteristics

Basic soil properties of the SRC were obtained in May 2011 (Table 1). In all soil layers, sand was the main grain size, predominantly the middle and the fine sand fraction. The total carbon (C_t) content was below 0.3% in all layers except for the plough layer (Ap1) at a depth of 0–40 cm, where the maximum total contents ($C_t = 1.3\%$, $N_t = 0.07\%$) were measured. This layer protrudes into the Ap2 layer with mixed tongue-shaped patterns.

The nutrient supply of the soil was analysed twice a year. In spring and autumn, five soil cores (volumetric sampling) were taken from each subplot and analysed for pH, C_t, total nitrogen (N_t), and the extractable potassium (K_{CAL}), phosphorus (P_{CAL}) and nitrogen (N_{min}) in the soil layers 0-30 cm and 30-60 cm. N_{min} (NH₄⁺ and NO₃⁻) was extracted from 50 g fresh soil in 200 ml of 0.0125 M CaCl₂. Ammonium and NO₃⁻ from the extracts were measured colorimetrically using the CFA SAN-SYSTEM (Skalar). Plant available content of P, K and Mg was measured from 5 g of the dried soil after extraction in 100 ml CAL solution. Phosphorus was measured using the CFA SAN-SYSTEM (Skalar), K and Mg using AAS 1100B (Perkin Elmer). The plant available elements phosphorus, potassium and magnesium were never limited during the study (data not shown). Seasonal differences of soluble N_{min} between the fertiliser treatments were calculated for the soil layer 0-60 cm applying a bulk density of 1.6 g cm $^{-3}$.

Nitrate Leaching

Nitrate output by leaching from the root zone of poplar was measured by self-integrating accumulators (SIA) provided by TerrAquat Consultants (http://www.terraquat.com/sia) [32]. The SIA consist of PVC cartridges of 0.1-m height and 0.1m diameter. They are filled with a mixture of quartz sand and ion exchange resin and equipped with a nylon net in the bottom to assure an undisturbed water flux through the SIA and to stabilise the filling in the cartridge. The SIA were inserted laterally beneath the undisturbed soil at the lower boundary of the system under study at a depth of 90 cm just below the main rooting zone of poplar. The SIA were installed in the fertilisation treatments 0, 50 and 75 kg N ha⁻¹. Three to four SIA were installed in each subplot. They remained in the soil for the duration of the sampling periods to accumulate the leached NO₃. After each sampling period (Fig. 2), the SIA were excavated and NO₃⁻ was extracted from the resin material using 1 M NaCl. Nitrate-N leaching rates for each SIA were calculated in kilogram N per hectare and sampling period. Average N leaching rates from May to October ("summer") and from November to April ("winter") were calculated for each subplot. Due to low NO₃⁻ leaching rates during summer, the measurement period was expanded to 12 months starting in October 2011.

Greenhouse Gas Emissions

Direct CO_2 and N_2O emissions from the soil were sampled in the four replicated subplots per fertiliser treatment (0, 50 and 75 kg N ha⁻¹ year⁻¹) using closed cylindrical chambers with a volume of 0.064 m³ (upper diameter 0.39 m, lower diameter 0.51 m, height 0.40 m). The gas chambers made of polyvinyl chloride consisted of opaque white chamber bodies that were placed on opaque grey bases which were inserted into the ground to a depth of 10 cm. Measurements were performed between 9 and 11 a.m. four times a week from June 2008 until December 2012. The chambers were located between planting rows, which were 1.8 m apart. Gases were sampled in 125-ml evacuated glass vials at the beginning and the end of the 90-min incubation period. During this period, cumulative CO_2 and N_2O emissions increased nearly linearly, which was

Table 1 Physico-chemical soil properties of the SRC at Potsdam in May 2011

Soil layer	Depth (cm)	Sand %	Silt %	Clay %	$C_t (g kg^{-1})$	$N_t(g\;kg^{-1})$	pH (CaCl ₂)	Bulk density (g cm ⁻³)
Ap1 + Ap2	0–40	77.8	17.2	4.9	12.60	0.69	6.71	1.54
		83.6	13.3	3.1	2.44	0.06	6.62	nd
Bv	40-60	85.7	11.9	2.4	0.47	0.03	6.35	1.65
Cv1	60–80	92.8	7.2	< 0.5	0.27	0.02	6.81	1.62
Cv2	80-100	93.6	5.8	0.6	0.19	0.02	6.77	1.65

nd not determined



checked before over a time of 150 min. Gas samples were analysed using a gas chromatograph (Shimadzu GC 14A) according to Hellebrand et al. [12] on the same day. Gas fluxes were calculated from the observed concentration changes in relation to chamber volume and ground surface area covered. The N_2O detection limit was 5 ppb. Fertiliser-induced N_2O conversion factors were calculated in the period of Jan. 01, 2009–Dec. 31, 2012 (n = 595) for each rate of fertilisation based on the difference between the mean values of the fertilised and the non-fertilised subplots [15].

Statistical Analysis

Mixed linear model analysis of the influence of several factors on NO₃⁻ leaching and CO₂ and N₂O fluxes were performed using the R statistical software [33] included in the RStudio program [34] and the R-package nlme [35]. For NO₃ leaching, seasonal data from summer 2008 to summer 2011 was included (that is four repetitions for summer and three repetitions for winter, Fig. 2). The response variable "NO₃" leaching" was log10-transformed after adding a constant of 0.1 to achieve normality of model residuals. The treatment variable "fertilisation" (0, 50, 75 kg N ha⁻¹ year⁻¹) and the additional predicting variables "season" (summer-winter), "rotation period", and the interactions between "fertilisation and season" were included as fixed effects. Measurements were repeated in time and, hence, their observations and residuals are not independent. To control for non-independence of residuals, we treated the subplots within blocks as random effects in the model. For NO₃ leaching, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Multiple comparisons of seasons and rotation periods were conducted with the Tukey-HSD function from the multcomp package [36]. For CO₂ and N₂O fluxes as response variables, a mixed effect model was established for the period June 2008-December 2012. This time covered three rotation periods. The treatment variable "fertilisation" and the additional predicting variables "rotation period", "weeks" and "air temperature" were included as fixed effects for the model. While the first three variables are of direct interest for testing the hypotheses, the "air temperature" was included as a confounding variable to improve the model. Multiple samples are taken over time from the same subplot. As single measurements were unevenly spaced in time, the exponential correlation (corExp) function was fitted in order to model temporal correlation. The selection of the correlation structure was based on Akaike's Information Criteria. For CO₂ and N₂O fluxes, visual inspection of residual plots did not reveal deviations from homoscedasticity or normality. The effect of fertilisation levels on N₂O emissions up to 30 days after application was tested with a two-factorial ANOVA model. The response variable was the natural logarithm of the N₂O emissions, and the factors were fertilisation rate (N=0, 50 and 75) and fertilisation period (years from 2008 to 2012) as well as the interaction of the factors. Heteroscedasticity between fertilisation periods was considered in the model. Differences between the fertilisation levels were then tested separately for every fertilisation period. All tests were performed at a significance level of $\alpha=0.05$. For gas emissions, multiple comparisons of rotation periods were conducted with the Tukey-HSD function from the multcomp package [36].

Results

Weather Conditions

From 2008 to 2012, annual means of air temperature and precipitation sum ranged between 9.2–11.1 °C and 464–663 mm, respectively (Fig. 3). The mean air temperature was 10.2 °C and the mean annual precipitation rate 557 mm between Jan. 01, 2008 and Dec. 31, 2012, indicating warmer and dryer conditions compared with the years before. The beginning of the vegetation period in April/May was often characterised by dry conditions. Just after planting at the end of April 2008, there was a long period of 7 weeks with a very low precipitation rate of 10 mm. As a result of the rainfall, there was a bulk atmospheric deposition of N with a mean input of 12.4 kg N ha⁻¹ year⁻¹ during the study period.

Soil Characteristics and Nutrient Supply

When the tree cuttings were planted in 2008, the N_{min} levels of the soil (0–60 cm) were low in all treatments with less than 5 kg ha⁻¹. One year after the poplar cuttings had been planted, the N_{min} supply increased and reached a level between 20 and 32 kg N ha⁻¹. N fertilisation did not result in significantly different supply of soluble N in the three fertiliser treatments. It could be shown for the whole study period that the soil under study provided optimum nutrition for poplar. The upper soil layer contained 0.56 g kg⁻¹ Mg, 0.19 g P_2O_5 kg⁻¹ and 0.12 g K_2O kg⁻¹ in May 2010. Therefore, no fertilisation with Mg, P and K was needed.

Root Development in Poplar Trees

The biomass of fine poplar roots increased in all fertiliser treatments from less than 0.5 t to 1.0–2.0 t dry matter per hectare from 2008 to 2012 (Fig. 4a). During the first year, the annual fine root productivity was very similar for the fertilisation rates with a mean value of 35 g DM m⁻² year⁻¹, which was not as high as reported by Berhongaray et al. [37] who found a fine root productivity of ca. 44 g DM m⁻² year⁻¹ in a poplar SRC in Belgium. Growth and development of fine roots seemed to be affected by the rate of fertilisation. Low



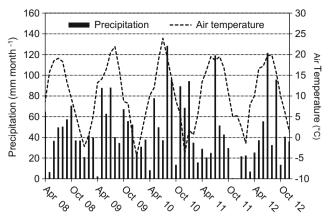


Fig. 3 Monthly mean air temperature and sum of precipitation in Potsdam during the period from April 01, 2008 to Dec. 31, 2012

rates of N fertilisation tended to yield the highest biomass of fine roots, an observation which corresponds with poplar pot experiments by Kruse et al. [38]. In the years 2010 and 2012 when the surface biomass was harvested, a stagnation of the fine root growth was observed. As shown for the root productivity, the N content of poplar fine roots (dry weight) were very similar for the fertilisation rates. During the first rotation, the N content ranged between 2.1 and 2.7% and during the second rotation between 1.4 and 1.8%. This corresponded to an N pool of 18–25 kg N ha⁻¹ year⁻¹ in summer 2012 (data not shown).

In 2009, the year after the young poplar trees were established, fine root ectomycorrhization did not exceed 1% of fine roots in all treatments, 0, 50 and 75 kg N ha⁻¹ year⁻¹ (Fig. 4b). However, 1 year later, the fine roots of all treatments

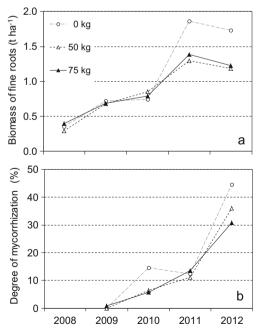


Fig. 4 Development of fine root dry weight biomass (a) and ectomycorrhization of fine roots (b) of poplar in a soil depth 0–40 cm and 0–10 cm, respectively

were associated with mycorrhiza, particularly the non-fertilised treatment. In the following years, the most intensive ectomycorrhization was observed in the non-fertilised treatment, with 45% in 2012. At the same time, the amount of ectomycorrhization ranged between 31 and 36% in the treatments with N fertilisation.

Nitrate Output by Leaching

The linear mixed effects model was applied to compare the influence of fertilisation, seasons and rotation periods on NO₃⁻ leaching. Not the "fertilisation" itself but only "season" and "rotation period" had a significant impact on NO₃⁻ leaching (Table 2, Fig. 5).

Multiple comparisons showed that NO_3^- leaching was low in summer with median values below 0.1 kg N ha⁻¹ but significantly higher values in winter ranging between 10.7 and 17.6 kg N ha⁻¹ (Fig. 5b). Taking all fertilisation levels into account, 5.8 kg N ha⁻¹ year⁻¹ was leached during the first 4 years of the poplar SRC (Table 3). According to IPCC [39], indirect N₂O emissions are obtained by applying a conversion factor of 0.75% yielding in a $CO_{2\text{equ}}$ of 13.0 kg ha⁻¹ year⁻¹. Leaching was also significantly higher during the first rotation compared with the second rotation period. The mean leaching rate of NO_3^- decreased in that time from 3.53 kg N ha⁻¹ half-year⁻¹ to 0.82 kg N ha⁻¹ half-year⁻¹ (Table 3).

N₂O emissions from the soil

Nitrous oxide fluxes changed over time under all fertilisation treatments of the poplar stand. The seasonal variability of the N_2O flux is shown in Fig. 6. Only during the first 2 years after the plantation was set up did N_2O emission exceed 30 μg m⁻² h⁻¹ several times, but after that it remained below this value.

For the whole study period with the mixed linear model applied, no significant effects of N fertilisation on N_2O flux were obtained (Table 4). Only if selected periods of 30 days after fertilisation are being considered, evidence is given for fertiliser-induced emissions of N_2O by significant differences in the second rotation period. By comparison with the zero N fertilisation, that was the case for the 50 kg and 75 kg N treatments in the years 2011 and 2012 and between 2010 and 2012, respectively (Fig. 7).

The mixed effects model revealed that the fixed effects "weeks" (time after planting and harvest) and "rotation period" influenced the N_2O fluxes significantly (Table 4). The correlation structure indicated a temporal autocorrelation of up to 2 days, indicating that the emissions on 1 day are correlated to those emissions on the 2 preceding sampling days. The continuous predictive variable "weeks" increased N_2O emissions by 0.004 (± 0.003 S.E.) kg N year⁻¹ per °C.



Table 2 Linear mixed effects model to test for the overall effect of the fixed effects on NO₃⁻ leaching

Fixed effect	Numerator	Denominator	F value	Pr>F
Fertilisation	2	6	1.94	0.2246
Season	1	68	165.62	< 0.0001
Rotation period	1	68	55.12	< 0.0001
Fertilisation x season	2	68	0.91	0.4066

 N_2O emissions were lowest in the second rotation period compared with the 1st and the 3rd rotation (Table 5). Averaged over all three rotations, the loss of N via N_2O was 0.61 kg $ha^{-1}~year^{-1}$ corresponding to 182 kg $CO_{2\rm equ}$. Considering the background N_2O emissions of non-fertilised subplots, the mean fertiliser-induced conversion factor of the SRC in the years 2009–2012 ranged between 0.14 and 0.18% in the poplar stand.

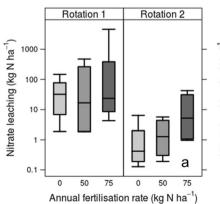
CO₂ Emissions from the Soil

During the study period with two rotation periods, CO_2 emissions followed an expected seasonal pattern, which was closely correlated with the air temperature (Table 6). However, interannual variations were found with enhanced CO_2 emissions in 2010 and 2012, which were the 2 years with harvest, compared with the preceding years 2009 and 2011, respectively (Fig. 8).

For the non-fertilised subplots, the annual mean emission rates in 2009/2010 and in 2011/2012 increased from 182 to 190 mg $\rm CO_2~m^{-2}~h^{-1}$ and from 185 to 211 mg $\rm CO_2~m^{-2}~h^{-1}$, respectively. The same pattern was observed for the fertilised subplot 75 kg N with an increase from 195 to 221 $\rm CO_2~m^{-2}~h^{-1}$ and from 168 to 224 m $^{-2}~h^{-1}$, respectively. $\rm CO_2~emissions$ tended to increase with the fertilisation rate, but there was no significant difference between the fertilised and the non-fertilised subplots as shown in Table 6.

The mixed effects model revealed that except for "fertilisation", all fixed effects influenced the CO₂ fluxes significantly (Table 6). The correlation structure indicated a temporal autocorrelation of up to 6 days. The effect of the

Fig. 5 Semi-annual NO_3^- leaching in poplar SRC on a logarithmic scale in relation to annual fertilisation rate plotted separately per rotation period (a) and per summer and winter sampling period (b). The crossbar within the box shows the median, the length of the box depicts the interquartile range and the fences mark the extremes if there are no outliers (n = 4)



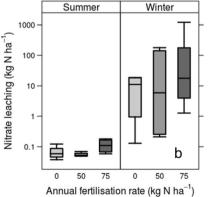


Table 3 Back-transformed least square means and standard errors of NO_3^- leaching (kg N ha⁻¹ half-year⁻¹) and results of paired *t* tests within effects

Effect	Level	Least square mean	Standard error
Season	Summer	0.56 a	1.21
	Winter	5.24 b	1.22
Rotation period	1 (after planting)	3.53 a	1.21
period	2 (after 1st harvest)	0.82 b	1.22

Different letters indicate significant differences ($\alpha = 0.05$ level)

continuous predictive variable "weeks" had a small negative effect of $-0.55~mg~CO_2~m^{-2}~h^{-1}~(\pm\,0.09~S.E).$ The confounding variable "air temperature" had a positive effect on CO_2 emissions with mean increases per unit of 7.15 mg $CO_2~m^{-2}~h^{-1}~(\pm\,0.21~S.E.).$ $CO_2~emissions$ increased significantly with the rotation period: rotation 1< rotation 2< rotation 3 (Table 7) with an emission rate increasing from 15,122 to 19,091 kg $CO_2~ha^{-1}~year^{-1}.$

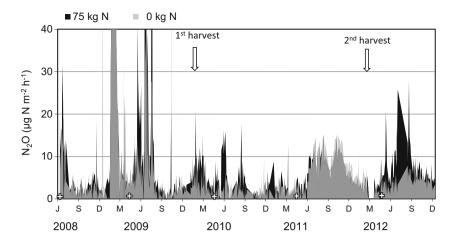
Discussion

Planting and Harvest Effects on Carbon and Nitrogen Dynamics in SRC

Before planting of poplar started, the field under study was used for agricultural production of annual crops. In April 2008, a young stock of winter rye was ploughed, which resulted in an input of organic matter to the soil. Degradation of this carbon pool was not measured in this study, but according to Möller et al. [40], it may be estimated to be around 20 kg N ha⁻¹ due to the N incorporation of young rye plants, which had been sowed the autumn before. The increased SOM mineralisation after ploughing following no-till grain management might have also led to an additional N source. High N_2O emission rates at the beginning of 2009 also in the nonfertilised subplots as well reflected an exclusive supply of N,



Fig. 6 Seasonal N₂O emissions from the soil of fertilised and nonfertilised poplar. Arrows and crosses indicate the time of harvest and fertilisation (75 kg N ha⁻¹ year⁻¹), respectively



which could not be fully taken up by the young plants. Due to this additional N supply through mineralisation of organic matter and the input of 12.4 kg N ha⁻¹ year⁻¹ by atmospheric N deposition, no N mineral fertiliser was needed during the first growing period of poplar. This observation is in line with results from Lasa et al. [41], who studied the influence of age in the efficiency of N fertilisation on poplar in the first year of growth. The authors concluded that N fertiliser use efficiency was highest at a late fertilisation time, when plants were well developed to absorb and to assimilate nitrogen. Thus, a surplus of N was high after fertilisation in our study, as indicated by enhanced N₂O emissions, which followed the time of fertiliser application (Fig. 8). However, with increasing biomass yield from one rotation period to the next, it may be expected that the need for N fertiliser will increase with the age of the SRC.

Enhanced CO₂ emissions in the poplar stand were recorded in 2010 and 2012, corresponding to years with harvest, compared with the preceding years 2009 and 2011 without harvest. This effect may have different causes: (i) as a consequence of the above-ground harvest, the soil is exposed to enhanced solar irradiation and warming during the following spring and summer, which results in higher mineralisation rates and (ii) it may be due to advanced decay of tree roots, which become inactive as long as the stems do not resprout. The latter was indicated by a stagnation of fine root biomass after harvest in 2010 (Fig. 4a) pointing to a stimulation of heterotrophic respiration. The opposite pattern was observed

Table 4 Linear mixed effects model to test for the overall effect of the fixed effects on N_2O fluxes for the time series from June 2008 until December 2012

Fixed effect	Numerator	Denominator	F value	Pr>F
Fertilisation	2	9	2.35	0.1506
Air temperature	1	8096	1.61	0.2048
Weeks	1	8096	6.71	0.0096
Rotation period	2	8096	16.93	< 0.0001

1 year later in 2011, when fine root biomasses reached their maximum particularly in the non-fertilised treatment. CO₂ emissions from the soil of the non-fertilised treatment were higher compared to the 75 kg N treatment nearly during the whole vegetation period of 2011 (Fig. 8). Therefore, it is most likely that in the year without harvest, autotrophic respiration was the dominating source of CO₂ as reflected by enhanced fine root biomass. Similar results were observed in an Italian study site. Abou Jaoude et al. [42] reported an increase of CO₂ emissions in a poplar stand with 3-year rotations after coppicing. However, in contrast to our results, the authors did not observe a change of heterotrophic respiration and reported only an enhanced autotrophic respiration. Beyond these two different pathways of CO₂ release, a significant increase of CO₂ emissions was found from one rotation period to the next (Table 7), which we attribute to the age of trees affecting the respiration intensity of the SRC.

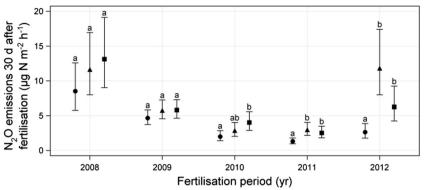
Driving Forces of NO₃ Leaching in SRC

Applied fertiliser is to be taken up by plants, but excessive fertiliser, particularly in the mobile form of the soluble NO₃⁻ ion, may easily be leached with the seepage water in sandy soils with low retention capacity for water and dissolved nutrients [15]. Such an effect of fertilisation on NO₃⁻ leaching, however, could not be found for the poplar SRC during the first 3.5 years after planting (Table 3). But in the winter, the average of N leaching rates (fertilised and non-fertilised treatments) exceeded the summer rates significantly by ten times. The same observation was made in a non-fertilised willow plantation in a drinking water catchment area in Northern Germany [43]. Leaching loss from a 5-year-old willow plot was 14.3 kg NO₃⁻ N ha⁻¹ during spring and only 2.0 kg NO₃⁻ N ha⁻¹ during the subsequent drainage period.

The dose-response effects from fertilisation treatments between 60 up to 360 kg N ha⁻¹ year⁻¹ had been reported by Sevel et al. [14] for a Danish willow SRC on sandy soils. Their results derived from water flux modelling using the NO₃⁻



Fig. 7 Nitrous oxide emitted from the soil of the poplar SRC in the period 0-30 days after fertilisation in the spring of each year. Error bars represent standard deviation of the mean (n = 17)



Fertilisation level (kg N ha⁻¹ yr⁻¹): ● 0 ▲ 50 **■** 75

concentration of the seepage water obtained by suction cups at 100 cm soil depth. Nitrogen leaching was generally low with rates between 1 and 7 kg N ha⁻¹ year⁻¹ as long as the N dose did not surpass 120 kg N ha⁻¹ year⁻¹. Higher doses up to 360 kg N ha⁻¹ year⁻¹ resulted in a leaching rate of 99 kg N ha⁻¹ year⁻¹.

Our study gives an insight into the nutrient dynamics of young poplar trees from the beginning of the SRC. In contrast to the first rotation period with relatively high N leaching rates, significantly less N was lost by leaching in the second rotation period. This pattern corresponds with the decrease of leached N in a Danish willow stand [44] and may be explained by a higher N demand and more efficient fertiliser use compared with the first rotation period. It is supposed that in the case of poplar, a mycorrhiza facilitates access to amino acids and oligopeptides from decomposed soil organic matter [45, 46], a substantial reason for low additional nutrition demand of poplar.

Only little information exists about the root biomass of SRC, because the subsurface biomass is not directly affected when the above-ground biomass is harvested. The turnover of coarse and stump roots, which belong to the greatest fraction of roots of SRC [47], has not been considered in this study. According to Berhongaray et al. [37], we focussed within the poplar rhizosphere on the development of fine roots with a diameter < 2 mm. Fine roots can be colonised with mycorrhizal fungi and underlie a seasonal dynamic, which may be boosted by the disruption caused by the surface biomass

Least square means and standard errors of N₂O flux estimates (μ g N m⁻² h⁻¹) and results of paired t tests within effects

Effect	Level	Least square mean	Standard error
Rotation period	1 (after planting)	8.35 a	0.50
1	2 (after 1st harvest)	4.91 b	0.48
	3 (after 2nd harvest)	7.56 a	0.78

Different letters indicate significant difference ($\alpha = 0.05$ level)



harvest. At the beginning of 2010 and 2012, when poplar plants were harvested for the first and second time, summer biomass of fine roots stagnated or were reduced compared with that prior to harvesting (Fig. 4a). This might have been a response to the loss of the above-ground biomass. One year later in 2011, when no external disruption occurred, there was a considerable increase of the fine root biomass in all fertiliser treatments. However, the degree of mycorrhization did not follow this pattern. It is obvious that the colonisation of newly developed fine roots by mycorrhizal fungi is not as fast as the growth of fine roots themselves.

Another interesting finding is the reduced degree of mycorrhization in those subplots which were fertilised with calcium ammonium nitrate. The highest degree of mycorrhization was detected in the non-fertilised subplots (Fig. 4b), pointing to a compensation of N supply. This observation coincides with studies of Baum et al. [48], who found reduced biodiversity of ectomycorrhizal fungi and reduced extraradical mycelium in the rhizosphere of Salix viminalis after fertilisation. The rhizosphere with mycorrhiza is known as that compartment with a high nutrient turnover [49]. With increasing age of the trees, a network of roots is formed, which enhances the retention of NO₃⁻ from one rotation period to the next and thereby reducing the leaching of NO₃⁻. Although the NO₃⁻ leaching from poplar SRC fields is low or negligible compared to reference fields with cereals [15, 50], the benefit of this production system is not fully recognised in agricultural practice yet.

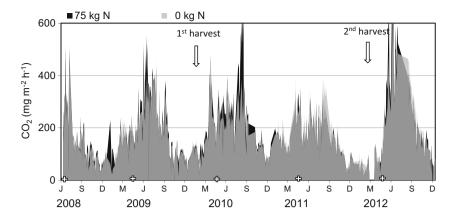
The ratio between the amount of annual N leaching (sum of summer and consecutive winter leaching) and annual N

Linear mixed effects model to test for the overall effect of the fixed effects on CO2 fluxes

Fixed effect	Numerator	Denominator	F value	Pr>F
Fertilisation	2	9	0.72	0.5116
Air temperature	1	8144	1230.87	< 0.0001
Weeks	1	8144	68.74	< 0.0001
Rotation period	2	8144	17.92	< 0.0001



Fig. 8 Seasonal CO₂ emissions from the soil of fertilised and non-fertilised poplar. Arrows and crosses indicate the time of harvest and fertilisation, respectively



fertilisation shows that up to 82% of the N fertiliser applied to poplar was lost by leaching (Fig. 9). The ratio declined over the years as a result of rising water and nutrient demand by the plants and because of enhanced biomass of fine roots, which improved the uptake of water and nutrients.

The amount of N leached during the summer periods was significantly lower than that leached during the winter periods. Although not measured in this study, the visual inspection of the dried soil during the sampling of the SIA in autumn substantiated low rates of seepage water. Consequently, evapotranspiration exceeded the precipitation during the growing season. Leaching in summer probably only occurs during heavy rainstorms followed by preferential flow [51] and therefore NO₃⁻ leaching could be neglected during the summer months. With decreasing evapotranspiration rates during autumn and winter, excessive NO₃ was leached with the percolation water. The amount of NO₃⁻ leaching during the winter period mostly depends on the NO₃⁻ surplus in the root zone and therefore is also an indicator for the N use efficiency of the treatment. Taking a groundwater recharge of 91 mm year⁻¹ as estimated by Balasus et al. [16] and leaching rates of 10.7-17.6 kg N ha⁻¹ during the winter periods of this study (Fig. 5b), then the calculated leachate concentration of N is in the range of 103–170 mg NO₃⁻ L⁻¹ according to the fertilisation level. This range represents the winter periods of the first 21/2 rotation periods of poplar. In contrast to the initial situation, when the leachate concentration was calculated only for the first rotation period with up to 292 mg NO₃⁻ L⁻¹ [16], the

Table 7 Least square means and standard errors of CO_2 flux estimates (mg CO_2 m⁻² h⁻¹) and results of paired t tests within effects

Effect	Level	Least square mean	Standard error
Rotation period	1 (after planting)	172.63 a	4.34
F	2 (after 1st harvest)	190.35 b	4.09
	3 (after 2nd harvest)	217.93 с	6.76

Different letters indicate significance $\alpha = 0.05$ level

situation was improved in the second and third rotation period. However, it can be predicted that the leachate concentrations resulting from fertilising the SRC with 75 kg N ha⁻¹ will not fulfil the threshold of 50 mg NO₃⁻ L⁻¹ fixed by the European Nitrate Directive yet. But it can be expected, that this goal will be achieved when the coppice crop has been fully established [11].

Driving Forces of GHG Emissions in SRC

Among the GHG emitted from agricultural operations, N₂O is the most potent, due to its high global warming potential, about 300 times higher than that of CO₂. One driving force of N₂O emission is the intensity of N fertilisation. Numerous studies have reported the relationship between N₂O emission and mineral and organic N fertilisation [52–54]. However, in this study, an effect of fertilisation on N₂O emissions could not be observed for the full study period, probably due to relatively low fertilisation rates of maximum 75 kg N ha⁻¹ with a high variability. Only for selected periods as for example 30 days following the N application as suggested by Hellebrand et al. [28], revealed significantly enhanced N₂O emissions from the soil.

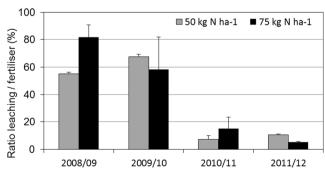


Fig. 9 Percentage of leached N fertiliser from the poplar rhizosphere expressed as ratio between leaching rate (sum of summer and following winter) and fertiliser application rate. Error bars represent standard deviation of the mean (n = 4)



There are still uncertainties about which further factors control the N₂O emission within perennial crops. As reported for CH₄ [55], soil moisture may also play a role in the emission of N₂O in energy crops, which however was not the focus of this study. But it could be shown that N₂O emissions were positively influenced by the number of weeks after planting in the 1st rotation period and harvesting in the 2nd and 3rd rotation periods, respectively. However, independently of the fertilisation level, they were highest during the first rotation period and particularly in its second year (2009), a relatively dry year with just 401 mm of annual rainfall. Thus, another factor was responsible, as already reported by Balasus et al. [16] for the first rotation period of this SRC. The high N₂O emissions during the first 2 years even in the non-fertilised subplots of both tree species were therefore rather the result of soil management. Ploughing rye into the soil before plantation installation in 2008 must have led to considerable storage of organic matter, yielding high availability and accessibility of nitrogen and other nutrients even 1 year later. All in all, the N₂O emissions were rather low and the mean values of the fertiliser-induced conversion factor were far below the threshold of 1% set by the IPCC [56].

Both, the soil preparation for planting as well as the harvest after 2 and 4 years have been shown to increase the GHG emissions of N₂O and CO₂, respectively. The main driving forces for the emission of CO2 in this study were air temperature, week after planting and harvest and the rotation period. However, the intensity of N fertilisation did not affect the emission of CO₂ over the whole study period, which is a further indication that the availability of N is not limited in the soil of poplar. In the initial phase of an SRC, a net C release may occur due to the soil management and the low growth of trees as reported by Don et al. [57]. However, after the second vegetation period, the biomass production of poplar usually leads to a net uptake of C with a storage rate of 4.5 t C ha⁻¹ on average in the wood [57]. Unlike Don et al. [57], we did not address a C balance in our approach and therefore we did not measure net C fluxes. Our focus was solely on CO₂ emission mainly driven by soil respiration, the greatest global C flux to the atmosphere [58]. In our study, the CO_2 emission from the soil decreased with time (weeks) after planting or harvesting, but increased from the first to the second and from the second to the third rotation period. It is noteworthy that under certain conditions, C may be enriched in the soil of SRC, as shown by Abou Jaoude et al. [42], Walter et al. [59] and Harris et al. [60]. Such C enrichment results from the decay of roots and litter and the turnover to soil organic carbon [42, 61]. Processes of C enrichment and C mineralisation in the soil seem to be very dynamic and dependent on the age and the management of an SRC, so that the duration of a study plays a crucial role in the final assessment of C sequestration. Based on long-term studies, a storage effect of C in the soil of woody energy crops can be suggested. In a comprehensive study, which considered

both, gross photosynthesis and respiratory fluxes from foliage, stem and soil, Verlinden et al. [62] reported that during the first year a newly established poplar SRC was a net source of carbon to the atmosphere. But already during the second growing period, the SRC became a significant carbon sink. Thus, well-established SRC have the potential to compensate or to offset GHG emissions in the energetic and the agricultural sector.

Perspectives for Adjusted Fertilisation of SRC

The highest risk of N leaching and N₂O emission occurs during the first 2 years within the first rotation period of poplar as long as no root system has been developed and the nutrient demand is still low. In our study, we could not prove over the first 4 years of the poplar SRC including 2½ rotation periods that both environmental effects become manifest up to a fertilisation rate of 75 kg N ha⁻¹ year⁻¹. In general, fertilisation enables growth stimulation and an increase in biomass yield. But this was not true during the first two rotation periods, when no significant differences in biomass yield of poplar were found between the fertilised and the non-fertilised treatments. In the second harvest, biomass yields were 5.4 t ha⁻¹ year⁻¹ in the zero and 6.3 t ha⁻¹ year⁻¹ both, in the 50 and 75 kg N treatment [63]. Based on these results, fertilisation of young poplar plants is not necessary and can be deferred during the first years. Another marginal agricultural land in southern Germany did not confirm these results [13], which may be explained by a higher mean annual precipitation rate of 790 mm year⁻¹ supporting a less restricted nutrient availability and a better growth. But the authors reported that the benefits of fertiliser-induced higher biomass yields did not counterbalance the negative environmental impacts as for example the leaching of NO₃, which had been identified as the main pathway for N loss from the sandy soil of our study site, primarily in the winter when high seepage rates emerged. This unavoidable seasonal constellation is one driving force behind the loss of N in the young SRC. However, agricultural management practice can be adapted to reduce the environmental impact, particularly the pollution of groundwater due to overfertilisation. The increase in NO₃⁻ concentrations has become the major problem affecting the quality of groundwater resources in many regions during recent decades [64]. Therefore, new strategies are needed to improve and to protect long-term groundwater resources [65]. Several options exist for solving this problem and ensuring the supply of high quality of drinking water. This study has shown that the production of biomass in SRC can be one option, since it has been proved to need relatively low amounts of N for growth. At least the initial phase of the SRC under study suggests that zero N fertilisation meets the N demand of young poplar trees. It is an environmentally sound management practice supporting



both, the production of renewable energy and the quality assurance of groundwater resources.

Conclusions

This study provides a better understanding of various environmental side-effects such as NO₃ leaching and the emission of N₂O and CO₂ caused by soil and crop management of SRC, most notably by fertilisation with mineral nitrogen. Up to a fertilisation rate of 75 kg N ha⁻¹ year⁻¹, the maximum rate in this study, there was only a tendency but no significant negative environmental effect of N fertilisation during the first three rotations of a poplar SRC. Nitrate leaching was reduced with increasing age of the trees, and hence increasing number of rotation periods. It became obvious that a well-established root system could compensate for N leaching and to some extent for N₂O emissions. The emission of CO₂ did not correspond to N fertilisation, but rather, soil temperature and harvest of the surface biomass were the abiotic drivers of carbon turnover in the rhizosphere. The absence of the fertiliser-induced environmental effects up to a fertilisation rate of 75 kg N ha⁻¹ year⁻¹ can contribute to the assessment of the sustainability of biomass production by SRC and may support decisions on fertiliser management of SRC during the first rotation periods.

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