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## Nitrate leaching and soil nitrous oxide emissions diminish with time in a hybrid poplar short-rotation coppice in southern Germany

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#### **Abstract**

Hybrid poplar short-rotation coppices (SRC) provide feedstocks for bioenergy production and can be established on lands that are suboptimal for food production. The environmental consequences of deploying this production system on marginal agricultural land need to be evaluated, including the investigation of common management practices i.e., fertilization and irrigation. In this work, we evaluated (1) the soil-atmosphere exchange of carbon dioxide, methane, and nitrous oxide  $(N_2O)$ ; (2) the changes in soil organic carbon (SOC) stocks; (3) the gross ammonification and nitrification rates; and (4) the nitrate leaching as affected by the establishment of a hybrid poplar SRC on a marginal agricultural land in southern Germany. Our study covered one 3-year rotation period and 2 years after the first coppicing. We combined field and laboratory experiments with modeling. The soil N<sub>2</sub>O emissions decreased from 2.2 kg N<sub>2</sub>O-N ha<sup>-1</sup> a<sup>-1</sup> in the year of SRC establishment to 1.1–1.4 kg N<sub>2</sub>O-N ha<sup>-1</sup> a<sup>-1</sup> after 4 years. Likewise, nitrate leaching reduced from 13 to 1.5–8 kg N ha<sup>-1</sup> a<sup>-1</sup>. Tree coppicing induced a brief pulse of soil N2O flux and marginal effects on gross N turnover rates. Overall, the N losses diminished within 4 years by 80% without fertilization (irrespective of irrigation) and by 40% when 40-50 kg N ha<sup>-1</sup> a<sup>-1</sup> were applied. Enhanced N losses due to fertilization and the minor effect of fertilization and irrigation on tree growth discourage its use during the first rotation period after SRC establishment. A SOC accrual rate of 0.4 Mg C ha<sup>-1</sup> a<sup>-1</sup> (uppermost 25 cm, P = 0.2) was observed 5 years after the SRC establishment. Overall, our data suggest that SRC cultivation on marginal agricultural land in the region is a promising option for increasing the share of renewable energy sources due to its net positive environmental effects.

*Keywords:* gross ammonification, gross nitrification, hybrid poplar short-rotation coppice, LandscapeDNDC, methane, nitrate leaching, nitrous oxide, soil greenhouse gas fluxes, soil organic carbon stocks

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### Introduction

The use of fossil fuels and fertilizers and land use change are responsible for the ongoing global climate change (IPCC, 2013) by increasing the atmospheric greenhouse gas (GHG) concentrations. The reduction in GHG emissions is in the political agenda (e.g., European Union 2020 strategy, EU, 2009), through the use of renewable energy sources, including biomass

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from crops (either conventional or dedicated, Don et al., 2012). However, the use of conventional crops (i.e., usually cultivated for food production) for bioenergy is controversial because it may result in a direct competition for food production (Wolf et al., 2003), increased fluxes of nitrous oxide (N<sub>2</sub>O) if crops are fertilized (Hellebrand et al., 2008) or the loss of C stocks due to vegetation clearings (Fargione et al., 2008). Even the use of dedicated bioenergy crops (otherwise not suitable for food production, e.g. Miscanthus spp, Populus spp) is likely to affect the availability of land for food and feed production, unless

marginal lands are targeted (Butterbach-Bahl & Kiese, 2013).

In this context, short-rotation coppices (SRC) with fast-growing tree species are an increasingly popular option for bioenergy production due to the potentially high yields of up to 24 Mg dry matter  $a^{-1}$  (Njakou Djomo et al., 2015), along with longer rotation periods than annual plants and lower fertilization needs than other energy crops (Karp & Shield, 2008). SRC are semiintensive managed systems (Don et al., 2012) that undergo substantially different management practices than conventional agriculture with respect to plant cover, fertilization rates and tillage practices (Davis et al., 2013). Therefore, changes in the GHG balance and modifications in the exchange of matter among the soil, atmosphere, and hydrosphere are expected when SRC are established on agricultural land (Palmer et al., 2014). Environmental changes do not necessarily occur instantaneously after land cover change, and detecting their temporal dynamics is crucial to develop more accurate GHG inventories (Poeplau et al., 2011), which should consider N<sub>2</sub>O and methane (CH<sub>4</sub>) emissions as well, given their strong global warming potentials compared with carbon dioxide (CO<sub>2</sub>) (Myhre et al., 2014).

The use of fertilizers at low rates (30-75 kg N ha<sup>-1</sup> a<sup>-1</sup>) is a common practice in SRC to increase plant biomass production through improved plant nutritional status (Scholz & Ellerbrock, 2002; Rewald et al., 2016). However, production of fertilizers is energy demanding (e.g., Kern et al., 2010) and fertilization incurs the risk of increased N losses due to enhanced nitrate (NO<sub>3</sub><sup>-</sup>) leaching (Zhou & Butterbach-Bahl, 2014) and N<sub>2</sub>O emissions (Butterbach-Bahl et al., 2013). Furthermore, fertilizers may decrease the sink strength of soils for atmospheric CH<sub>4</sub>, due to the inhibitory effect of ammonium (NH<sub>4</sub><sup>+</sup>) in methanotrophy (Steudler et al., 1989). Irrigation is used in SRC to improve water availability of the trees (Calfapietra et al., 2003), but watering changes soil moisture, which is a major driver for N<sub>2</sub>O emissions (Butterbach-Bahl et al., 2013). In this context, a few case studies have analyzed poplar SRC as sources and sinks for atmospheric N2O and CH4 (Balasus et al., 2012; Zona et al., 2013a,b; Walter et al., 2015; Zenone et al., 2016). However, there is almost no information on the microbial processes driving the soil-atmosphere fluxes of these GHGs (Lteif et al., 2010) and the extent to which they are affected by fertilization. Thus, a comprehensive analysis linking the microbial processes that drive soilatmosphere GHG exchange and the determination of how these processes are affected by SRC is crucial.

For our investigation, two widely used hybrid poplar clones, Monviso (Sixto *et al.*, 2014) and Max4 (Balasus *et al.*, 2012), were established on a marginal agricultural land in southern Germany, comprising three

management treatments: control (nonfertilized, nonirrigated), irrigated and fertigated (fertilized and irrigated). Herein we aimed to evaluate the environmental consequences of a newly established hybrid poplar SRC on marginal agricultural lands during 5 years of cultivation, comprising a three-year rotation period and two years after the first coppicing. In particular, we (1) quantified the soil-atmosphere CO2, N2O and CH4 fluxes in the plantation using a combination of automated chamber measurement campaigns and processbased modeling; (2) assessed the SOC changes after five years of SRC establishment by repeated soil sampling; (3) estimated the gross microbial ammonification and nitrification rates during one vegetation period by means of the <sup>15</sup>N pool dilution technique; and (4) estimated the NO<sub>3</sub> leaching below the active rooting zone by the use of suction cups and modeling. We hypothesized that (i) soil N<sub>2</sub>O emissions, microbial N turnover rates and NO<sub>3</sub> leaching would decrease due to the highly efficient N uptake of hybrid poplar SRC. We expected these changes in the N cycle to be evident within the first rotation period (3 years) after SRC establishment. Furthermore, we hypothesized that (ii) SOC levels would increase following the establishment of SRC; and that (iii) soil N2O emissions, microbial turnover and leaching would be enhanced due to fertilization and harvesting because both interventions imply an increase in the substrate availability for soil microorganisms.

#### Materials and methods

Site description

The experimental site is located near the town of Sigmaringen (48°6′23″N, 9°14′37″E) in the SW-German cuesta landscape with Jurassic limestone covered by tertiary loam residuals forming the bedrock geology. The landscape in this region has a fragmented structure, with agricultural land primarily in areas of 1-10 hectares with alternating commercial forest (mainly comprising Picea abies (L.) H. Karst. and Fagus sylvatica L.). The site itself is located at approximately 630 m a.s.l. The mean annual temperature is 7.2 °C, and the mean annual precipitation is 790 mm (446 mm in the growing season). The experimental area has an agricultural site quality index of 37 (in German Ackerzahl, Blume et al., 2010), indicating marginal growing conditions for arable crops (Aust, 2012; Schweier & Becker, 2013). Prior to the establishment of the hybrid poplar SRC, the site was under conventional agriculture for decades. The crop rotation scheme included wheat, barley, oats, and forage grasses.

Soil properties at the experimental site were determined by excavating four randomly located soil pits (1.25 m  $\times$  1.25 m  $\times$  0.80 m) in April 2009, immediately before the poplar cuttings were planted. The soil exhibited a clear plow horizon (30 cm deep). The soil bulk density ranged from 1.3 to 1.6 g cm<sup>-3</sup>. The soil texture ranged from silty clay in the plow horizon to clayey

silt in deeper layers (Table 1). The soil was identified as a Leptosol (FAO, 2006).

#### Experimental design

In April 2009, the area was grubbed, harrowed and treated with Roundup® (Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany) (Birmele et al., 2015). Immediately after this treatment, the site was planted with hybrid poplar cuttings. Two commercial poplar clone types were used: Max4 (Populus maximowizcii A. Henry x P. nigra L.) and Monviso (P. x generosa A. Henry × P. nigra L.). Planting was performed in rows spaced 250 cm apart; the in-row tree spacing was 50 cm (8000 stems ha<sup>-1</sup>). Each clone covered a continuous area of approximately two ha (Fig. S1), which was further split according to management treatments: (1) no treatment or control, (2) irrigation and (3) fertigation (irrigation and fertilization). In the initial year (2009), no fertilizer was applied because it was assumed that the site had residual fertility from the previous conventional agricultural use. Irrigation water and fertilizers were applied during the growing seasons of 2010, 2011, and 2012 (approximately April-October). Each treatment included four replicates in a randomized plot design, resulting in 24 plots, each of which consisted of six rows with a length of approximately 100 m (ca. 1000-1200 trees per plot).

The irrigated and fertigated plots were equipped with a drip irrigation system (Netafim Germany GmbH, Frankfurt a.M., Germany) consisting of polyethylene pipes installed along the tree rows. A pump located inside a well provided the water to the pipes. In the irrigation treatment, water was distributed automatically to the plots whenever the soil water tension (monitored by tensiometers) dropped below -150 hPa. The amount of water released from the well to each individual irrigation plot was recorded using a water meter, and monthly readouts permitted the quantification of the cumulative amount of water added within a specific period. There were slight but nonsignificant differences in the amount of water received by the plots (data not shown). The complementary water in the irrigated plots amounted to 157, 7, and 127 mm in 2010, 2011, and 2012, respectively. Fertigated plots received the same amount of water as the irrigation plots within each specific period. Dissolved NH<sub>4</sub>NO<sub>3</sub> was added to the irrigation water; accounting for 10 kg N ha<sup>-1</sup> for each fertigation event. At monthly intervals during the growing season, each fertigated plot was manually fertigated by opening a valve from the water tank until the corresponding amount of water with the dissolved fertilizer had been distributed. Fertigated plots were fertilized four times (2010 and 2011) or five times (2012) per year. On March 1st, 2012, the trees were machine harvested and chopped. The poplar stems were cut at a height of approximately 10 cm to allow resprouting. One control plot was left uncut for comparison purposes.

### Measurements of soil-atmosphere greenhouse gas exchange

The soil GHG fluxes were measured using a mobile automated chamber system. This measurement system allowed for the simultaneous determination of N2O, CH4, and CO2 soil-atmosphere fluxes with a 2-h time resolution. Transparent chambers  $(0.5 \text{ m} \times 0.5 \text{ m} \times 0.15 \text{ m})$  opened and closed automatically, driven by compressed air. The headspace of each chamber was sampled sequentially at different time intervals after chamber closure (0, 15, 30, and 45 min). The headspace air was pumped at a rate of 200 ml min<sup>-1</sup> into an automated sampling system, which finally injected an aliquot every 3 min into a gas chromatograph (Shimadzu GC 14, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector for the determination of CH<sub>4</sub> and an <sup>63</sup>Ni electron capture detector for the determination of N2O. CO2 was determined using an infrared gas analyzer (Li-Cor, Lincoln, NE, USA). Upstream of the sampling system, water vapor was removed using a Perma Pure dryer (Perma Pure Llc, Toms River, NJ, USA). Prior to the injection of an aliquot into the gas chromatograph, CO2 was removed by means of an Ascarite column (sodium hydroxide-coated silica). Calibration gas was added every 2 h. Rates of soil-atmosphere exchange of each GHG were calculated based on the linear change of its concentration within the headspace after chamber closure. Air temperature (2 m) and soil temperature (10 cm depth) at each plot were recorded every minute with PT100 probes (IMKO GmbH, Ettlingen, Germany).

Four intensive monitoring campaigns were conducted in the SRC to obtain temporally high resolved soil-atmosphere GHG exchange fluxes. In 2009 (17-27th of August), 12 chambers were deployed in control areas because fertigation and irrigation had not yet started. Due to the malfunctioning of one of the chambers, the results from 11 chambers are presented. In 2010 (5th of May to 21st of July), the control, irrigation and

Table 1 The mean  $\pm$  standard error (n = 4) soil features of the experimental site near Sigmaringen, southern Germany, in April

Depth (cm)	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	CaCO <sub>3</sub> (g kg <sup>-1</sup> )	Bulk density (g cm <sup>-3</sup> )	Sand (g kg <sup>-1</sup> )	Silt (g kg <sup>-1</sup> )	Clay (g kg <sup>-1</sup> )
0–10	$20.9 \pm 3.8$	$2.3\pm0.4$	$11.0 \pm 11.7$	$1.13\pm0.02$	279 ± 8	$485\pm25$	236 ± 24
10-20	$19.9 \pm 1.1$	$2.1\pm0.1$	$1.3 \pm 1.3$	$1.34 \pm 0.04$	$263 \pm 4$	$495\pm14$	$242\pm17$
20-30	$15.6 \pm 3.0$	$1.7 \pm 0.3$	$1.5 \pm 2.2$	$1.41\pm0.08$	$278\pm14$	$506 \pm 6$	$217\pm19$
30-40	$10.1 \pm 1.3$	$1.2\pm0.1$	$2.3 \pm 4.5$	$1.51 \pm 0.06$	$272\pm4$	$525 \pm 23$	$204\pm20$
40-50	$7.4\pm0.6$	$0.9\pm0.1$	$0.3 \pm 0.5$	$1.54\pm0.04$	$259\pm9$	$503 \pm 19$	$239\pm20$
50-60	$5.9 \pm 0.5$	$0.8\pm0.0$	$7.8 \pm 14.8$	$1.53 \pm 0.03$	$249\pm15$	$449 \pm 33$	$302\pm40$
60-70	$4.9\pm0.2$	$0.6\pm0.0$	$51.0 \pm 77.0$	$1.58\pm0.12$	$248\pm30$	$430 \pm 12$	$323 \pm 38$
70-80	$3.6\pm0.4$	$0.6\pm0.0$	$58.3\pm114.5$	$1.63\pm0.09$	$257\pm28$	$427\pm23$	$316\pm27$

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fertigation treatments were monitored (n=4). In autumn 2011 (5th of September to 19th of October), only the control and fertigation plots were monitored (n=3). In 2012, a campaign was undertaken from the 22nd of February to the 27th of March, during which the chambers were deployed in the control plots, and the effect of coppicing on the soil GHG fluxes was investigated by comparing harvested and nonharvested plots (n=3). Repeated-measures anovas were conducted using the daily values from each chamber as individuals. Time was considered as a within-subjects factor, and the treatments were used as the between-subjects factor. Significance was established at P=0.05.

# Gross ammonification and nitrification rates and microbial biomass

The 15N pool dilution technique was used on intact topsoil cores collected from the control and fertigation plots for the estimation of gross rates of ammonification and nitrification as described in Dannenmann et al. (2006). The method is based on the analysis of the artificially 15N-enriched signature of the NH<sub>4</sub><sup>+</sup> pool, which is diluted due to gross ammonification, or the NO<sub>3</sub> pool, which is diluted due to gross nitrification (Kirkham & Bartholomew, 1954; Davidson et al., 1992). Six adjacent intact soil cores (5 cm in diameter, 4 cm deep) per spatial replicate were collected and immediately transported to the laboratory, where soil analysis started within 18 h after sampling. Two soil cores were labeled with 30% K<sup>15</sup>NO<sub>3</sub> for the determination of gross nitrification rates and two soil cores were labeled with 30%  $(^{15}\mathrm{NH_4})_2\mathrm{SO_4}$  for the determination of gross ammonification rates; the other two cores were used for the determination of soil microbial C and N content and the soil water content. For isotope application, a custom-built multiinjector (Gütlein et al., 2016) was used to achieve the best compromise between the opposing goals of (1) homogenous labeling, (2) maintaining the soil structure, (3) reproducibility of labeling, (4) minimal water addition, and (5) minimal leakage from the bottom of the soil cores. Labeling of the analyzed soil samples was performed using 30 micro-injections of 0.3 ml for every soil core at three different soil depths, equaling an addition of 2–3 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> sdw (soil dry weight) or mg NO<sub>3</sub><sup>-</sup>- $N kg^{-1} sdw$ .

Following an equilibration phase of approximately 1 h after isotope labeling, the soil from half of the cores was extracted using  $0.5~M~K_2SO_4~(1:2~soil:solution,~w:v)$ , whereas the remaining cores were incubated for almost two days in darkness at mean daily field temperature before being extracted in the same way. The  $^{15}N$  enrichment of the  $NH_4^+$  and  $NO_3^-$  pools was quantified by the diffusion of  $NH_4^+$  and  $NO_3^-$  on acid filter traps and subsequent isotope ratio mass spectrometry (Dannenmann *et al.*, 2009). The  $NH_4^+$  and  $NO_3^-$  concentrations were determined by colorimetry in a commercial laboratory (Landwirtschaftliches Labor Dr. Janssen, Gillersheim, Germany). The calculations of the gross ammonification and nitrification rates were conducted using previously described equations (Kirkham & Bartholomew, 1954).

Soil microbial C and N contents were quantified using the chloroform fumigation-extraction (FE) technique (Brookes et al.,

1985) as described in detail by Dannenmann et al. (2009) within one day after soil sampling. A homogenized fresh soil sample was divided into two subsamples weighing approximately 75 g each. The first subsample was extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> (1:2 soil:solution, w:v). The second soil subsample was fumigated with chloroform and subsequently extracted. Soil extracts from both the control and the fumigated samples were frozen before being analyzed for total chemically bound N and total dissolved organic C contents using a chemoluminescence analyzer coupled to a TOC analyzer (DIMATOC® 2000, Dimatec Analysentechnik GmbH, Essen, Germany), and for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> concentrations as described above. The soil microbial C and N contents were calculated from the difference between the fumigated and control samples without the application of correction factors to provide conservative estimates of the active part of the microbial biomass (Perakis & Hedin, 2001).

These soil sampling and analysis routines were conducted at fortnightly to monthly intervals to investigate seasonal variations. Cumulative turnover rates were obtained for the period March–December 2011 based on linear interpolation between the sampling dates. The effects of tree harvesting on the gross rates of the N transformation processes were assessed by the estimation of gross ammonification and nitrification rates prior to and after coppicing in winter 2012. Statistically significant differences (P < 0.05) between the control and fertigation samples with respect to the gross turnover rates, soil inorganic N contents and soil microbial C and N contents were explored using Student's t-tests.

#### Nitrogen leaching

The inorganic N concentrations in the soil water were measured at different depths in the control and fertigation plots. For this purpose, suction cups (UMS GmbH, Munich, Germany) were installed at soil depths of 30 and 100 cm (n = 6 for each treatment and depth). A suction cup consisted of a porous ceramic tip inserted into the soil at the specific depth and gastightly connected via a plastic tube to a glass bottle. The bottle was subjected to pressure using a vacuum pump to allow for water sampling. The bottles were replaced fortnightly, and the soil water was analyzed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents as described above. This approach allowed for the estimation of the mean inorganic N contents (i.e., the sum of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in the soil solution for the periods between bottle replacement. The concentrations measured at a soil depth of 30 cm were considered to be those available for plant N uptake. The N present in the soil water solution at a depth of 100 cm was considered not available for plant uptake because this soil depth is well below the active rooting zone. The NH<sub>4</sub><sup>+</sup> contents were <0.1 mg l<sup>-1</sup> and are therefore not included here. Nitrogen leaching losses were estimated by multiplying the measured concentrations with the modeled soil water fluxes (see below).

#### Changes in soil organic carbon stocks

To assess temporal changes in the topsoil SOC contents as affected by the cultivation of hybrid poplar SRC, two detailed assessments of the SOC stocks were performed (April 2009 and

March 2014) using a repeated soil sampling approach. Eightyone spatially explicit points located at the intersections of a 10m × 10-m regular grid were sampled at soil depths of 0-5 and 20-25 cm. Soil samples were sieved (2 mm), air dried and analyzed for total C (DIN ISO-10694), total N (DIN ISO 13878) and carbonate content (VDLUFA Method IA 5.3.1). Total organic C was calculated as the difference between total and inorganic C. Additional soil samples were collected using stainless cylinders, and soil bulk density was determined after oven drying at 105 °C for 24 h. First, the SOC stocks (Mg C  $ha^{-1}$ ) were calculated for each sampled soil layer by using the SOC concentrations, soil bulk density, depth of the soil layer and gravel content (Aalde et al., 2006). Furthermore, SOC stocks for the uppermost 25 cm at each intersection were calculated by assuming that the SOC stocks changed linearly with depth between the 0-5-cm and the 20-25-cm layers. Finally, the results from individual locations were linearly extrapolated to generate a spatially continuous map of the distribution of the SOC stocks using SIGMAPLOT 12.5 (Systat Software, Inc., Chicago, IL, USA). The SOC concentrations in each soil layer and the SOC stocks in the top 25 cm of the soil profile were compared between sampling years (2009 and 2014) using Student's t-test; the individual results from each intersection (n = 81) were used.

Modeling soil-atmosphere greenhouse gas exchange rates and nitrate leaching using LandscapeDNDC

The soil CO2 and N2O effluxes and the hydrology of the rotation period of the hybrid poplar SRC were simulated using LandscapeDNDC with a physiological process model previously evaluated for poplar (Werner et al., 2012). LandscapeDNDC is a modular modeling platform that combines process-based models of C, N, and water cycling within soil and plants for site and regional scale applications (Haas et al., 2013). Processes and state variables are considered in a vertically structured one-dimensional column that extends from the top of the canopy to the rooting depth or a given soil depth.

LandscapeDNDC requires general initial site, soil, and vegetation information as well as climate, air chemistry, and management data as driving forces. Detailed vertical profile information on the soil characteristics was available from direct measurements (humus type, clay content, organic C and N contents, bulk density, saturated conductivity, stone content, pH, water-holding capacity and wilting point) and was obtained in increments of 2 cm throughout the profile to a depth of 100 cm. In addition, daily climate data (temperature, precipitation, radiation) and management inputs (planting, fertigation, irrigation and harvesting) were available from measurements and records at the site level. The CO2 concentration of the air was obtained from a nearby meteorological station (Deutsche Wetter Dienst; DWD Station Sigmaringen-Laiz). N-deposition and air chemistry details were estimated from general sources for the region (Schaap et al., 2015). In-depth descriptions of the functions involved in plant physiology, SOC mineralization, N<sub>2</sub>O emissions and NO<sub>3</sub> leaching can be found elsewhere (Li et al., 2000; Stange et al., 2000; Chirinda et al., 2011; Grote et al., 2011a,b; Kiese et al., 2011). The parameter set for the plant physiological processes was previously tested for poplar (Werner et al., 2012).

Four model input files were used to estimate the effect of soil heterogeneity on the LandscapeDNDC outputs of the GHG fluxes and water leaching. These input files differed with respect to soil characteristics, which were measured in individual pits (Table 1). Two-way ANOVA was used to compare the GHG fluxes and water leaching results between years and treatments. Daily soil GHG fluxes gained from the automatic chamber measurements and the use of LandscapeDNDC were compared for the periods with field observational data.

#### **Results**

Soil nitrous oxide emissions

The average soil N2O fluxes in August 2009 (17th to 27th) were 30.0  $\pm$  7.5  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> and showed a high spatial and temporal variability (Fig. S2). The mean daily values from 17th to 24th of August ranged between 10 and 46 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>, before a rain event triggered a short-lasting N2O soil emission pulse of  $41-84 \mu g$  N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>; even peak emissions >120  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> were measured in some chambers. The values decreased to 30–50  $\mu g$  N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> by the following day. The soil N2O fluxes measured in spring and summer 2010 were lower than those measured in 2009, and no significant differences among treatments were observed (25.2  $\pm$  4.7, 21.9  $\pm$  1.8 and  $27.7 \pm 6.8 \ \mu g \ N_2O-N \ m^{-2} \ h^{-1}$  for the control, irrigated and fertigated plots, respectively, Fig. S3). The soil N<sub>2</sub>O fluxes during fall 2011 were within or near the detection limit of our measurement system (ca. 5 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>, Fig. S4), and there were no differences between the control and fertigated plots. A slight diurnal pattern was observed from the end of September to the beginning of October (Fig. S5): a weak N2O emission peak occurred at midday or in the afternoon (2-4 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>), and modest N<sub>2</sub>O uptake rates were recorded during the early evening and at night (-2 to  $-4 \mu g N_2 O-N m^{-2} h^{-1}$ ).

In February 2012, no initial differences in soil N<sub>2</sub>O emissions (prior to tree harvesting) were observed between harvested (6.0  $\pm$  4.2  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>) and nonharvested plots (1.6  $\pm$  3.3  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>, Fig. 1). The day after coppicing, the soil N<sub>2</sub>O emissions were  $13.3 \pm 4.5$  and  $1.6 \pm 4.2 \mu g N_2 O-N m^{-2} h^{-1}$  from the harvested and nonharvested plots respectively (P < 0.05). The mean soil N<sub>2</sub>O effluxes during the monitoring period after harvesting (2nd to 28th of March) from the harvested plots and nonharvested plots were 17.4  $\pm$  6.1 and  $9.2 \pm 0.9 \ \mu g \ N_2 O-N \ m^{-2} \ h^{-1}$  respectively (P = 0.24).

The soil N<sub>2</sub>O fluxes from the hybrid poplar SRC modeled using LandscapeDNDC showed clear seasonal

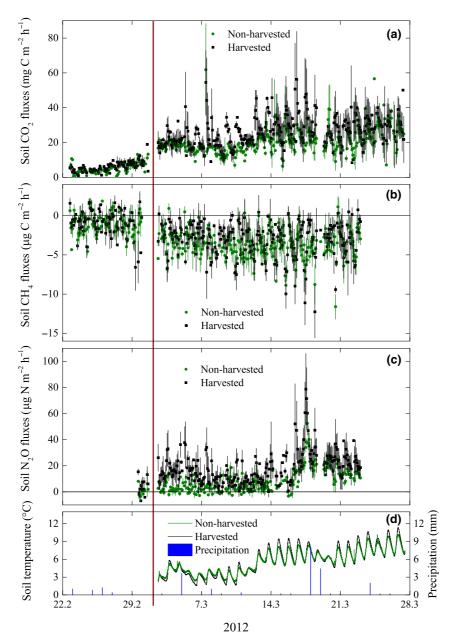


Fig. 1 Soil-atmosphere  $CO_2$  (a),  $CH_4$  (b), and  $N_2O$  (c) exchange rates in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2012. Symbols represent mean values  $\pm$  standard error (n = 3). The lower panel (d) shows the soil temperature at a depth of 5 cm (°C) and the daily precipitation (mm). The vertical red line indicates the tree harvest.

dynamics (Fig. 2). Precipitation events triggered the soil  $N_2O$  emissions, especially when the air temperatures were above 10 °C. Despite some divergence between measured and modeled soil  $N_2O$  effluxes (Fig. S6), LandscapeDNDC showed a decrease in the cumulative soil  $N_2O$  fluxes over the rotation period—from 2.2 kg N ha<sup>-1</sup> a<sup>-1</sup> in 2009 to 1.1–1.4 kg N ha<sup>-1</sup> a<sup>-1</sup> in 2012—and a modest effect of fertigation or irrigation on the soil  $N_2O$  fluxes (Table 2), in line with the automatic chamber measurements. Variability in the model output

with respect to the soil  $N_2O$  effluxes resulting from the different model inputs ranged from 10 to 15% for the period of analysis (2009–2012). Simulated soil  $N_2O$  fluxes were significantly higher than measured values in August 2009, when measured fluxes were highly variable in space and time (coefficient of variation of 75% Fig. 2, Fig. S2). In autumn 2011, modeled soil  $N_2O$  emissions were 8–18  $\mu g$   $N_2O$ -N m $^{-2}$  h $^{-1}$  while measured soil  $N_2O$  fluxes were close or below the detection limit of the automatic system (Fig. 2, Fig. S4).

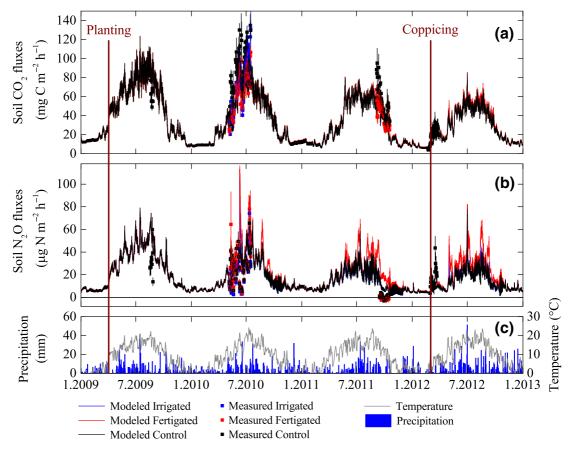


Fig. 2 Soil-atmosphere exchange rates of CO<sub>2</sub> (a) and N<sub>2</sub>O (b) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, as modeled using LandscapeDNDC (mean  $\pm$  standard error, n = 8, daily values) and measured with automatic chambers (mean  $\pm$  standard error, n = 3–6). Panel (c) shows mean daily air temperature (°C) and daily precipitation (mm). Brown vertical lines indicate planting and harvesting respectively. Note that irrigation and fertigation started in 2010.

Table 2 Mean annual cumulative soil N<sub>2</sub>O and CO<sub>2</sub> emissions and NO<sub>3</sub> leaching over the first rotation period of SRC on former arable land as modeled with LandscapeDNDC. Due to spatial heterogeneity four different soil initialization set-ups were used. Results correspond to mean values ( $\pm$  standard deviation, n = 8) of two poplar clones (Monviso and Max4). Irrigation and fertigation started in 2010. Different capital and small letters denote significant differences (P < 0.05) between years and treatments, respectively

Annual emissions	Treatment	2009	2010	2011	2012
Soil $N_2O$ emissions (kg N $ha^{-1} a^{-1}$ )	Control Irrigation	$2.20 \pm 0.14 \text{ A}$ n.a.	$1.57 \pm 0.20 \text{ B}$ $1.59 \pm 0.20 \text{ A}$ $1.70 \pm 0.21 \text{ A}$	$1.24 \pm 0.26$ C $1.24 \pm 0.26$ B $1.45 \pm 0.45$ AB	$1.13 \pm 0.21 \text{ bC}$ $1.17 \pm 0.19 \text{ bB}$ $1.37 \pm 0.19 \text{ aB}$
Soil $CO_2$ emissions (Mg C $ha^{-1}$ $a^{-1}$ )	Fertigation Control Irrigation	n.a. 3.93 ± 0.41 A n.a.	$2.87 \pm 0.21 \text{ A}$ $2.87 \pm 0.31 \text{ B}$ $2.90 \pm 0.31 \text{ A}$	$1.43 \pm 0.43 \text{ AB}$ $2.68 \pm 0.23 \text{ B}$ $2.67 \pm 0.22 \text{ A}$	$2.33 \pm 0.18 \text{ C}$ $2.34 \pm 0.18 \text{ B}$
$NO_3$ leaching (kg N ha <sup>-1</sup> a <sup>-1</sup> )	Fertigation Control Irrigation Fertigation	n.a. 13.1 ± 2.02 A n.a. n.a.	$2.93 \pm 0.30 \text{ A}$ $3.8 \pm 1.1 \text{ bB}$ $4.6 \pm 1.2 \text{ bA}$ $8.1 \pm 1.5 \text{ aA}$	$2.77 \pm 0.20 \text{ AB}$ $1.8 \pm 0.9 \text{ bC}$ $1.8 \pm 0.9 \text{ bB}$ $5.0 \pm 1.4 \text{ aB}$	$2.48 \pm 0.17 \text{ B}$ $1.5 \pm 0.7 \text{ bC}$ $1.9 \pm 0.8 \text{ bB}$ $8.2 \pm 1.0 \text{ aA}$

Soil-atmosphere methane exchange rates

The soil was a net weak sink of atmospheric CH<sub>4</sub> throughout all monitoring periods, without significant differences between control, irrigated or fertigated plots across the monitoring periods. In August 2009, the mean  $CH_4$  uptake rates were  $-7.7 \pm 1.0 \mu g$   $CH_4$ -C  $m^{-2} h^{-1}$  (Fig. S2). In 2010, the net soil CH<sub>4</sub> uptake rates ranged from  $-4.9 \pm 2.6$  for the control plots to  $-4.0 \pm 1.8 \ \mu g \ CH_4$ -C m<sup>-2</sup> h<sup>-1</sup> for the irrigated plots, with fertigated plots showing intermediate values (Fig. S3). Later in 2010, the net soil CH<sub>4</sub> uptake rates dropped to virtually 0 after a precipitation event in mid-June and then steadily increased until mid-July, when the CH<sub>4</sub> fluxes stabilized at  $-10~\mu g$  CH<sub>4</sub>-C m $^{-2}$  h $^{-1}$  (Fig. S3). In autumn 2011, the control and fertigated plots had virtually identical net soil CH<sub>4</sub> uptake rates (-7.7 and  $-7.5~\mu g$  CH<sub>4</sub>-C m $^{-2}$  h $^{-1}$ , respectively, Fig. 2). In 2012, the soil CH<sub>4</sub> uptake rates were low both before and after the tree harvesting (Fig. 1), and no difference between harvested and nonharvested plots was observed ( $-3.1\pm0.6~\rm vs.$   $-3.6\pm0.1~\mu g$  CH<sub>4</sub>-C m $^{-2}$  h $^{-1}$ ).

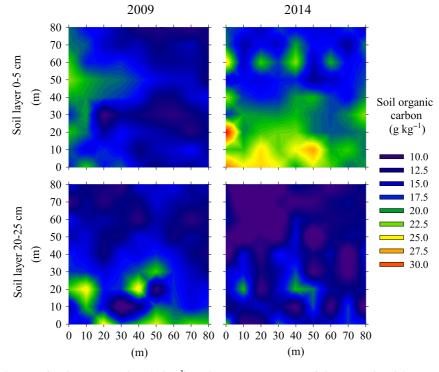
#### Soil carbon dioxide effluxes

The measured soil  $CO_2$  effluxes in August 2009 were about 70 mg  $CO_2$ -C m<sup>-2</sup> h<sup>-1</sup> (Fig. S2), with daily and temporal variation accounting for 20–25%, and 50%, respectively. In 2010, measured soil  $CO_2$  effluxes were higher in the control plots than in the fertigated plots (89  $\pm$  1 vs. 67  $\pm$  3 mg  $CO_2$ -C m<sup>-2</sup> h<sup>-1</sup>, respectively; P=0.02), with irrigation plots showing intermediate values (73  $\pm$  7 mg  $CO_2$ -C m<sup>-2</sup> h<sup>-1</sup>, Fig. S3). Higher soil  $CO_2$  effluxes in the control plots were also observed in autumn 2011 compared with the fertilized plots (54  $\pm$  5 and 42  $\pm$  8 mg  $CO_2$ -C m<sup>-2</sup> h<sup>-1</sup>, respectively, Fig. S4). In February 2012, the soil respiration rates were very low and did not differ

significantly between the plots that were subsequently harvested and those that remained uncut (5.6  $\pm$  0.4 and 6.1  $\pm$  1.1 mg CO<sub>2</sub>-C m $^{-2}$  h $^{-1}$ , respectively, Fig. 1). The coppicing resulted in minor, nonsignificant effects on soil CO<sub>2</sub> efflux over the three weeks following coppicing (25  $\pm$  5 and 20  $\pm$  1 mg CO<sub>2</sub>-C m $^{-2}$  h $^{-1}$  for the harvested and nonharvested plots, respectively).

Soil organic carbon concentration and soil organic carbon stocks

In 2009, the SOC concentrations were virtually identical for the 0–5 and the 20–25 cm soil layers (20.1  $\pm$  3.0 and 20.3  $\pm$  3.8 g kg<sup>-1</sup>, respectively). In 2014, a decrease in the SOC concentrations with increasing depth was observed (24.2  $\pm$  3.7 and 16.8  $\pm$  4.2 g kg<sup>-1</sup> in the 0–5 and 20–25 cm soil layer, respectively; P < 0.01). Consequently, the SOC concentrations in 2014 were significantly higher at 0–5 cm and significantly lower at 20–25 cm compared with 2009 ( $P < 10^{-3}$  for both cases, Fig. 3). A similar pattern was observed for total N (Fig. S7). The soil bulk density did not differ significantly between sampling years in the 0–5 cm soil layer (1.22  $\pm$  0.10 and 1.30  $\pm$  0.09 g cm<sup>-3</sup> in 2009 and 2014, respectively; P = 0.12) and the 20–25 cm soil layer (1.38  $\pm$  0.11 g cm<sup>-3</sup> in both years). The SOC stocks in



**Fig. 3** Spatial distribution of soil organic carbon (g kg<sup>-1</sup>) in the uppermost 5 cm of the mineral soil (upper panels) and at a 20 to 25 cm depth (lower panels) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2009 (left panels) and 2014 (right panels).

the uppermost 25 cm of the soil profile were estimated as  $65.7 \pm 11.8$  in 2009 and  $67.8 \pm 9.5$  Mg C ha<sup>-1</sup> in 2014; the difference (2.1 Mg C ha<sup>-1</sup>) was not statistically significant due to the high spatial variability (P = 0.23).

#### Gross nitrogen turnover rates

The average gross ammonification rates in 2011 were  $1.3 \pm 0.3 \ \text{mg N} \ \text{kg}^{-1} \ \text{sdw day}^{-1}$  in the control plots and  $2.0 \pm 0.4$  mg N kg<sup>-1</sup> sdw day<sup>-1</sup> in the fertigated plots (Fig. 4). Results from August, September and November were significantly different between treatments (P = 0.01-0.05). The cumulative ammonification rates for the 2011 growing season were 157  $\pm$  11 and  $236 \pm 19 \text{ kg N ha}^{-1} \text{ a}^{-1}$  for the control and fertigated plots, respectively (P < 0.01, Fig. 4a).

The average gross nitrification rates in 2011 were  $0.6 \pm 0.2$  and  $0.8 \pm 0.4$  mg N kg<sup>-1</sup> sdw day<sup>-1</sup> for the control and fertigated plots respectively. The cumulative gross nitrification rates were virtually identical for the control and fertigated plots (58  $\pm$  3 and 59  $\pm$  6 kg N  $ha^{-1} a^{-1}$ , respectively, Fig. 4a).

The extractable inorganic N contents in the topsoil remained between 1 and 5 mg kg<sup>-1</sup> sdw for both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> throughout 2011 (Fig. 4b) and were not altered by fertigation. The soil microbial C and N concentrations in the topsoil were low in general and were not affected by fertigation and peaked in July (Fig. 4c). The C:N ratio of the soil microbial biomass was narrow, with values ranging from 4 to 7.

A precoppicing analysis in February 2012 showed no significant differences between the uncut control plots and the not-yet-harvested plots with respect to gross N turnover, extractable soil N contents, and soil microbial biomass (Table 3). Similar postharvest comparisons revealed a significantly higher soil NO<sub>3</sub> content in the cut plots one day after harvesting (Table 3). Two months after harvesting, a marginal (P = 0.1) trend of higher nitrification rates and higher soil inorganic N content was observed in the cut plots (Table 3).

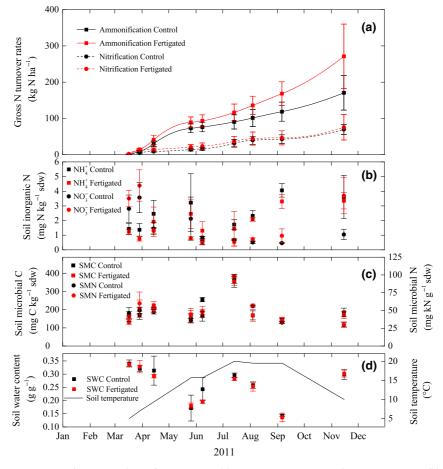


Fig. 4 Cumulative gross ammonification and nitrification rates (a); mean ammonium and nitrate contents (b); mean microbial biomass C and N contents (c); and mean soil water content and soil temperature during incubation in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany. All values are the mean  $\pm$  standard error (n = 9).

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**Table 3** Soil inorganic nitrogen contents, gross microbial N turnover rates and microbial C and N contents in 0–4 cm soil depth as affected by tree harvesting (March 1st 2012) at a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany. Mean ( $\pm$  standard error, n = 9) gross rates of microbial ammonification and nitrification as determined by the  $^{15}$ N pool dilution technique and microbial C and N as determined by the fumigation extraction technique

Date	Plot	Soil water content (g g <sup>-1</sup> )	Soil ammonium (mg N kg <sup>-1</sup>	Soil nitrate sdw)	Gross ammonification (mg N kg <sup>-1</sup> sdw	Gross nitrification day <sup>-1</sup> )	Microbial C (mg C kg <sup>-1</sup> sdw)	Microbial N (mg N kg <sup>-1</sup> sdw)
29-02	(Harvested)	$0.30\pm0.01$	$3.16 \pm 0.37$	$1.11 \pm 0.33$	$0.79\pm0.14$	$0.33\pm0.02$	$215\pm46$	$20 \pm 5$
	Nonharvested	$0.29\pm0.05$	$3.27\pm0.32$	$1.23\pm0.01$	$0.88\pm0.25$	$0.19\pm0.15$	$229\pm47$	$24 \pm 7$
02-03	Harvested	$0.29\pm0.01$	$3.89\pm0.59$	$1.72 \pm 0.30*$	$2.17 \pm 1.05$	$0.69\pm0.53$	$197\pm46$	$22 \pm 6$
	Nonharvested	$0.31\pm0.01$	$4.46\pm0.48$	$0.82\pm0.12^*$	$1.20\pm0.23$	$0.58\pm0.20$	$164\pm61$	$18 \pm 7$
14-03	Harvested	$0.29\pm0.01$	$2.65\pm0.14$	$1.71\pm0.24$	$1.79 \pm 0.38$	$0.71\pm0.29$	$129 \pm 33$	$24\pm4$
	Nonharvested	$0.29\pm0.01$	$3.07\pm0.50$	$1.99\pm0.52$	$1.39 \pm 0.35$	$0.40\pm0.06$	$147\pm19$	$21\pm2$
02-05	Harvested	$0.28\pm0.02$	$1.45\pm0.18$	$3.04\pm0.93$	$2.02\pm0.21$	$0.88\pm0.30$	$283 \pm 10$	$31 \pm 4$
	Nonharvested	$0.22\pm0.01$	$0.96\pm0.47$	$2.19\pm1.08$	$2.29 \pm 0.50$	$0.22\pm0.19$	$245\pm19$	$28\pm4$

sdw, denotes soil dry weight.

### Nitrate leaching

The NO<sub>3</sub> concentrations in the soil solution at 30 cm were usually higher than those at a depth of 100 cm; this was illustrated by both the modeled and measured data. The LandscapeDNDC outputs revealed higher soil NO<sub>3</sub> concentrations in response to the precipitation and fertigation events (Fig. 5). Generally, the mean NO<sub>2</sub> concentrations decreased from 2009 toward the end of the rotation period in spring 2012. Accordingly, the losses declined from approximately 13 kg N  $ha^{-1}$   $a^{-1}$  at the beginning of the rotation period to approximately 1.5 kg N ha<sup>-1</sup> a<sup>-1</sup> in the control and approximately 8 kg N ha<sup>-1</sup> a<sup>-1</sup> in the fertilized plots at the end of the first rotation period (Table 2). The differences between years were statistically significant, as were the NO<sub>3</sub> leaching values between the control and fertigation plots and between the irrigation and fertigation plots (Table 2).

#### Discussion

Effect of hybrid poplar SRC establishment on the soil nitrogen cycling

In our study, the hybrid poplar SRC was established on a site that was previously used for conventional agricultural production, involving rotations of cereals with forage grass (personal communication with the landowner). This type of management involves application of mineral fertilizer at rates exceeding 100 kg N ha<sup>-1</sup>, which generally results in mean soil  $N_2O$  emissions in the range of 5 to 10 kg N ha<sup>-1</sup> a<sup>-1</sup> (e.g., Flessa *et al.*, 1995) due to the high availability of mineral N in the soil for nitrification and denitrification

processes, yielding N2O as a sub-product (Butterbach-Bahl et al., 2013). The observed N2O emission rates in our study (approximately 30  $\mu g$   $N_2O-N$   $m^{-2}$   $h^{-1}$  in summer, or 1–2 kg N ha<sup>-1</sup> a<sup>-1</sup>) were within the range reported for other poplar or willow SRC in Europe (Hellebrand et al., 2008; Balasus et al., 2012; Drewer et al., 2012; Zona et al., 2013b; Zenone et al., 2016), and lower than the expected values for conventional agricultural sites in Germany (7.4 kg N<sub>2</sub>O-N ha<sup>-1</sup> a<sup>-1</sup>, Boeckx & Van Cleemput, 2001), southern Germany (> 6 kg  $N_2O-N$  ha<sup>-1</sup> a<sup>-1</sup>, Freibauer, 2003), the Baden-Württemberg state  $(3.5-4.5 \text{ kg N}_2\text{O-N ha}^{-1} \text{ a}^{-1}, \text{ Bouw-}$ man, 1996; Dechow & Freibauer, 2011; Stehfest & Bouwman, 2006) or the Sigmaringen county (5-7 kg N<sub>2</sub>O ha<sup>-1</sup> a<sup>-1</sup>, Henseler & Dechow, 2014), where this study took place.

Both field measurements and LandscapeDNDC outputs showed a steady decline in the soil N<sub>2</sub>O emissions, which were reduced by nearly 50% in the 4-year period (Table 2, Fig. 2). There are two reasons that soil N<sub>2</sub>O emissions decreased following the establishment of SRC. First, N fertilizer rates in SRC are reduced, or may not even occur (Don et al., 2012), compared with common agriculture practice (from 100-150 to <75 kg N ha<sup>-1</sup> a<sup>-1</sup>). Second, poplar SRC clones for biomass production have a substantial N demand for wood biomass production (Luo & Polle, 2009); estimations for our SRC based on biomass yields (Schweier et al., 2016) and from the measurement of wood and bark N contents (Kreuzwieser, personal communication) indicates a N demand for wood growth up to 20 kg N ha<sup>-1</sup> a<sup>-1</sup>. The combination of these two factors (low N input and high N uptake) resulted in strong decreases in soil mineral N concentrations and low N availability for soil microbes. The microbial resupply of mineral N via

<sup>\*</sup>Significant differences between treatments as determined by t-Student tests (P < 0.05).

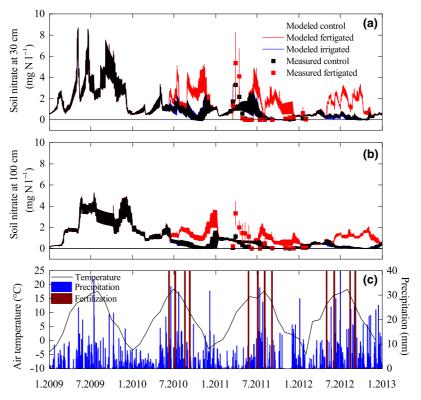


Fig. 5 Daily modeled (lines, mean  $\pm$  standard error, n = 8) and measured (squares, mean  $\pm$  standard error, n = 6) values of the soil nitrate concentrations at depths of 30 cm (a) and 100 cm (b) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany. Brown vertical lines indicate fertigation events (10 kg N ha<sup>-1</sup> each).

ammonification and nitrification was substantial during the growing season (ca. 200 and 60 kg N ha<sup>-1</sup>, respectively) in our poplar SRC, though the cumulative gross N turnover was smaller than that reported for forests (Rennenberg & Dannenmann, 2015). In this context, the low and even further decreasing mineral N concentrations observed in the topsoil, along with the high biomass increments in 2010 and 2011 (Schnitzler et al., 2014) suggest an increasing dominance of the poplars in terms of soil N uptake over time (Lteif et al., 2010), decreasing the N available for production of N2O via microbial nitrification and denitrification.

We hypothesized that fertilization would increase N<sub>2</sub>O emissions (Hellebrand et al., 2008), but this could not be confirmed by our field measurements. Fertilization rates in our study were between 2 and 4 times lower than those applied in the study of Hellebrand et al. (2008), and fertilization, applied with droplet irrigation next to the stems, was split in up to five applications across the growing season. This together has probably limited the occurrence of periods with surplus mineral N in the soil that otherwise would be available for nitrification and denitrification. Mineral fertilization increased the gross ammonification rates, likely by stimulating the activity of mineralizing bacteria and the enzyme production rates (e.g., Stone et al., 2012), but this had no further effects on the gross nitrification rates (Fig. 4a). Tree biomass growth (Schnitzler et al., 2014; Schweier et al., 2016) or N concentrations in biomass (Kreuzwieser, personal communication) were only marginally affected by fertilization, indicating a very limited effect of N fertilization on SRC performance (Scholz & Ellerbrock, 2002; Balasus et al., 2012), at least during the first rotation period when soil N stocks are still high due to the former use of fertilizers (Liberloo et al., 2009).

The values of NO<sub>3</sub> leaching were typical for agricultural sites (e.g., Di & Cameron, 2002) in the year of establishment of the hybrid polar SRC, but in the range of unfertilized forests in Germany (Kiese et al., 2011) in the rest of the years (Table 2). The fertilization effect on the NO<sub>3</sub> leaching was clear, even in view of some differences between measured and modeled NO<sub>3</sub> concentrations (Fig. 5). This finding shows that fertilization can substantially counteract the overall positive effect of SRC on the ecosystem N balance via the promotion of undesired hydrological N losses. NO<sub>3</sub> leaching is problematic in the study region because NO<sub>3</sub><sup>-</sup> transported in the fissured aquifer of the limestone bedrock can cause rapid karst water contamination.

Effect of hybrid poplar SRC establishment on the soil carbon cycling

Bioenergy production can lead to increasing SOC stocks if annual crops are converted into perennial energy crops (Poeplau et al., 2011; Don et al., 2012), as is the case in the present investigation. Arable lands are characterized by the presence of a plow horizon with a relatively homogeneous abundance of SOC resulting from the incorporation and mixing of crop residues through tillage. Two main factors may have contributed to the enhanced SOC levels in the uppermost 5 cm of the topsoil within the 5 years of SRC establishment; firstly, the cessation of plowing in 2009, which prevented artificial homogenization of the plow horizon, and secondly, the high above- and belowground litter fall C input rates resulting from higher biomass production and the potentially high quality of the poplar litter fall (e.g., Badre et al., 1998). We observed a not statistically significant SOC gain of approximately 0.4 Mg C ha<sup>-1</sup> a<sup>-1</sup> from 2009 to 2014, which is within the range of other reported results (Don et al., 2012; Rytter, 2012), and suggests that the use of SRC with hybrid poplar in agricultural lands is not associated with net SOC releases during the first rotation period. Still, we appeal to the necessity of conducting long-term studies to overcome the spatial variability of the SOC concentrations (15-20%). The extrapolated CH<sub>4</sub> uptake rates for the investigated hybrid poplar SRC (0.5–1.0 kg CH<sub>4</sub>-C ha<sup>-1</sup> a<sup>-1</sup>) are similar to the values for cultivated land worldwide (0.9 kg CH<sub>4</sub>-C ha<sup>-1</sup> a<sup>-1</sup>, Dutaur & Verchot, 2007), indicating that the effect of hybrid poplar SRC on soil-atmosphere CH<sub>4</sub> exchange rates are minor importance (Don et al., 2012; Drewer et al., 2012).

Increased respiration rates due to N input have been reported (e.g., Contosta *et al.*, 2011), which are in contrast to our observations. The increase in available N in the soil may have caused a shift in the relative C distribution among belowground components (Maier *et al.*, 2004), suppressed CO<sub>2</sub> emissions from the soil (Butnor *et al.*, 2003), or facilitated stabilization mechanisms of soil organic matter (Janssens *et al.*, 2010). LandscapeDNDC was not able to capture the diminishing effect of N fertilization on soil respiration (Table 2), showing that adjustments to the plant and soil C modules might be necessary.

Effect of coppicing on the greenhouse gas fluxes

We expected a dramatic imbalance in the competition between plants and soil microorganisms as well as rhizosphere priming effects after removal of the aboveground vegetation, which may lead to the accumulation of mineral N in the soil and in turn, to enhanced soil  $N_2O$  emissions. Surprisingly, we did not observe such pronounced harvest effects on soil microbial activity. The gross nitrification rate and the soil  $NO_3^-$  concentrations tended to increase slightly (these effects were not significant), and the overall effect on  $N_2O$  fluxes was as little as 200 g N ha $^{-1}$ . This minor effect is likely due to the high resprouting capacity of the poplar clones, which reduces nutrient losses and seems to be an efficient strategy for the remobilization and securing of nutrients in the plant-soil system of SRC (Scholz & Ellerbrock, 2002; Luo & Polle, 2009), as also suggested by the absence of significant changes in soil respiration after harvesting. On the other hand, some  $NO_3^-$  leaching may have occurred following harvesting, preventing further denitrification and  $N_2O$  losses.

Environmental consequences of the use of hybrid poplar SRC on a marginal arable land in southern Germany

The establishment of hybrid poplar SRC on marginal agricultural land sharply decreased soil  $N_2O$  emissions and  $NO_3^-$  leaching within the first rotation period of 3 years, likely due to reduced microbial availability of soil inorganic N after increased hybrid poplar N uptake and no or low N fertilization rates. Upon our results, fertilization use is discouraged, at least during the first rotation period after conversion into SRC, because fertilization enhanced the  $NO_3^-$  leaching rates and had only a minimal stimulation effect on tree growth. Coppicing provoked only slight and short-lasting increases in N losses and microbial turnover rates. The results indicate that SRC systems are able to maintain or even increase the SOC stocks compared with the previous conventional agricultural use in a time span of 5 years after SRC planting.

In the frame of the increasing demand for bioenergy, the establishment of SRC with fast-growing hybrid poplar clones on marginal agricultural land is a promising option and, in view of the environmental positive effects of SRC observed in this study, it seems that this woody biomass production system is more efficient and sustainable in terms of N use than intensive agriculture. Still, further studies are needed to investigate whether conclusions obtained here hold true in another climatic areas if we are to increase the share of renewable energy sources in Europe.

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## **Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

- **Figure S1**. Aerial view of the hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in summer 2011.
- **Figure S2.** Soil-atmosphere exchange rates of  $CO_2$  (a),  $CH_4$  (b), and  $N_2O$  (c) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2009.
- **Figure S3.** Soil-atmosphere exchange rates of  $CO_2$  (a),  $CH_4$  (b), and  $N_2O$  (c) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2010.
- **Figure S4.** Soil-atmosphere exchange rates of  $CO_2$  (a),  $CH_4$  (b), and  $N_2O$  (c) in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2011.
- Figure S5. Details on the soil-atmosphere exchange rates of  $N_2O$  in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany, in 2011.
- **Figure S6.** Mean daily measured vs. corresponding LandscapeDNDC-modeled values of soil N<sub>2</sub>O (a) and CO<sub>2</sub> (b) effluxes in a hybrid poplar short-rotation coppice near Sigmaringen, southern Germany.
- **Figure S7.** Spatial distribution of total nitrogen (g g<sup>-1</sup>) in the uppermost 5 cm of the mineral soil (upper panels) and at a 20 to 25 cm depth (lower panels) in a poplar short-rotation coppice plantation near Sigmaringen, southern Germany, in 2009 (left panels) and 2014 (right panels).