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# Drainage and nitrate leaching assessed during seven years under perennial and annual bioenergy crops

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## Abstract

Sustainable bioenergy crops must contribute not only to the production of renewable energy but also to maintaining or restoring water resource and quality. The aim of this study was to quantify water drainage and nitrate leaching under perennial (*Miscanthus* × *giganteus* and switchgrass), “semi-perennial” (fescue and alfalfa) and annual (sorghum and triticale) bioenergy crops managed with two N fertilisation rates. Soil water and mineral N contents were measured twice a year during seven consecutive years. These measurements were used to initialize the STICS model which simulated in turn the amounts of drained water and nitrate leached below 210 cm. Semi-perennial crops produced less drainage than annual crops (64 *vs.* 133 mm $yr^{-1}$) despite a similar biomass production. Perennial crops resulted in an intermediate drainage (90 mm $yr^{-1}$) but a greater biomass production. The drainage was negatively correlated with biomass production for perennial and annual crops. Perennial crops exhibited much higher water use efficiency than the other species. Nitrate concentration in drained water was low for all crops, most often less than 20 mg $NO_3$ $l^{-1}$. It was lower for perennials than other crops, except for miscanthus on the first year of measurement. However, the comparison of model outputs with nitrate concentrations measured in subsoil after five years indicated that the peak of nitrate produced after miscanthus establishment was subsequently recovered by the crop in deep layers (below 210 cm). Perennial bioenergy crops have potential for restoring water quality but may decrease groundwater recharge in deep soils or dry climates.

## Introduction

Nitrogen (N) excess in water is a major concern in Europe as it poses direct threat to human and aquatic ecosystems [1]. Nitrate concentrations in rivers, lakes, aquifers and coastal water are high in many regions, mainly as a result of diffuse pollution from agriculture [2]. Nitrate in drinking water can harm human health and the N enrichment of aquatic ecosystems contributes to toxic algal blooms, water anoxia and biodiversity loss [2]. It also results in indirect nitrous oxide ($N_2O$) emissions through denitrification in riparian zones and hydrological networks [3]. The European Union has adopted several regulations aiming at protecting waters such as the Nitrate Directive (Directive 91/676/EEC) and the Water Framework Directive (Directive 2000/60/EC). In this context, “good agricultural practices”, including improvement of N fertilisation practices and introduction of catch crops, have been promoted to reduce N leaching. Nevertheless, their implementation would not be sufficient to meet the requirements of European regulations in large areas of intensive arable farming such as the Seine Basin in France [4] and greater changes in agricultural systems might be necessary [5].

The use of biomass as renewable carbon for bioenergy, biomaterials or biochemicals is expected to contribute to the energy transition in response to the challenges of climate change and depletion of fossil resources [6]. However, the development of biofuels produced from conventional food crops has raised concerns about their energy and greenhouse gas (GHG) balance, other environmental consequences and competition for food production [e.g. 7,8,9]. New conversion technologies and biorefineries allow converting non-food crops into biofuels or biomaterials [10,11]. Perennial C4 crops such as miscanthus and switchgrass are viewed as promising bioenergy crops because of their high biomass production with low nutrient requirements [12-14]. These crops and other perennials such as short rotation coppices or C3 grasses are thought to have other environmental benefits such as reduced nitrate losses [15-17]. Land conversion from arable cropping systems to perennial bioenergy crops could therefore help to improve water quality at the catchment scale [18]. However, perennial crops could also consume more water than annual crops because of their higher biomass production, longer growing season and deeper root systems [15,17]. A high water consumption would reduce the amount of water drained and therefore the groundwater recharge [19]. A sustainable bioenergy crop production must therefore consider jointly the amount of water drained and the nitrate concentration in drained water.

Experimental comparisons of perennial and annual bioenergy crops regarding drainage and N leaching are scarce in the literature. Pugesgaard et al. [17] compared winter wheat, grass-clover (mixture of ryegrass and clover) and willow managed for bioenergy production over three years in a sandy loam soil in Denmark. They found a lower drainage under grass-clover and willow than under winter wheat. Nitrate concentration was much lower under perennial crops (12-31 mg $NO_3$ $l^{-1}$) than under winter wheat (76 mg $NO_3$ $l^{-1}$), showing the capacity of perennial crops to significantly reduce N losses compared to annual crops. Nitrogen leaching was also measured under miscanthus and switchgrass in comparison with conventional arable crops [15,20]. These studies showed lower nitrate losses under perennial C4 crops than under annual crops. However, the first year or the first two years following miscanthus establishment have been shown to present a higher risk for N leaching than the subsequent years [16,21] and N fertilisation of perennial crops could increase N leaching [21,22].

In an earlier paper [23], we measured soil water consumption of various perennial and annual bioenergy crops along the growing season in a long-term experiment. The objective of the present study was to quantify drainage and nitrate leaching over seven years in the same experiment as affected by crop species, biomass production and N fertilisation level. Our approach consisted in combining measurements of soil water and mineral nitrogen contents with modelling to calculate water and nitrate fluxes.

## Materials and methods

### *Site and experimental design*

The long-term experiment called “Biomass & Environment” was established in 2006 at the INRA experimental station in Estrées-Mons, northern France (49.872°N, 3.013°E). The soil is a Haplic Luvisol [24]. Detailed soil characteristics are given by Ferchaud et al. [23]. Before 2006, the field had been cultivated for many years with annual crops and the previous crop was winter wheat following spring pea.

The experiment was initiated to study biomass production and the environmental impacts of a wide range of bioenergy crops. It includes eight rotations: four with C4 perennial crops (monocultures), two with C3 "semi-perennial" crops (destroyed every two or three years) and two with C3/C4 annual crops (Table 1). The perennial crops are miscanthus (*Miscanthus*×*giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in February (L). The semi-perennial crops are tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (× *Triticosecale* Wittmack). The experiment also includes two nitrogen treatments, low N (N-) and high N (N+), with fertiliser-N rates depending on the crops (Table 1). The rationale for defining the N rates was explained by Cadoux et al. [14].

The 2.7 ha field was divided into two parts in order to facilitate cropping operations and limit competition between plants due to differences in canopy height: (i) a split-block design in the west part for perennial crops with "rotations" in the main plots (miscanthus E, miscanthus L, switchgrass E, switchgrass L) and N fertilisation rates in the subplots (N- and N+), and (ii) a split-plot design in the east part for the other crops with rotations in the main plots (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilisation rates in the subplots (N- and N+). Both parts include three replicate blocks and 24 subplots of 360 $m^2$ each (Online Resource 1).

After winter wheat harvest in 2005, the field was mouldboard ploughed in early December and left bare during winter. Miscanthus was planted in April 2006 (1.5 rhizome $m^{-2}$) and switchgrass sown in June 2006 (seed rate = 15 kg $ha^{-1}$). In 2006, perennial crops were not harvested because of the low biomass production during the first year of growth. Their aboveground biomass was cut and left on soil surface. Semi-perennial crops were sown in 2006, 2009, 2011 and 2014, usually in April. Before sowing, the previous crop (alfalfa or fescue) was destroyed in late autumn with a cultivator and a disc harrow in 2008 and mouldboard ploughed in 2010 and 2013. Fescue and alfalfa were harvested in two or three cuttings depending on years, with the last cut in October. Annual crops were tilled superficially (12-15 cm deep) without inversion ploughing. Sorghum was sown in late May and harvested in late September. Triticale was sown in mid-October and harvested in late July or early August. A catch crop was sown every year in late August or early September between triticale and sorghum (oats in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2013). The N fertiliser was applied as UAN solution (urea ammonium nitrate). Perennial crops received a single annual application in late April. Fescue received N fertiliser at the beginning of each cycle of growth but seedling crops were not fertilised before the first cut, so that the total N rate varied between years. Sorghum was fertilised before sowing (late May) and triticale in March and late April. The experiment did not receive irrigation, except in May 2011 for semi-perennial and annual crops (58 mm in total) to facilitate establishment during a drought period. Sowing, harvest and fertilisation dates for each crop are given in Online Resource 2.

### *Climatic data*

Climatic data were obtained from an automatic weather station located on the experimental site. Over the period 2006-2014, the mean annual temperature was 10.6 °C, annual global radiation was 4614 MJ $m^{-2}$, annual rainfall ($P$) and Penman potential evapotranspiration ($PET$) were 699 and 715 mm respectively. The water balance ($P$ - $PET$) between April and October varied widely between years, from -160 mm in the wettest year 2013 to -433 mm in the driest year 2009 (Online Resource 3). The water balance between November and March was on average 191 mm and varied between 152 and 217 mm.

### *Crop production*

The aboveground biomass at harvest was measured for each crop. Immediately before harvesting, plants were collected manually in small subplots (between 2.5 and 21.6 $m^2$ depending on crop species) and weighed. All individual cuttings of semi-perennials were measured and summed up to obtain annual production. Details about sampling methodologies are given by Cadoux et al. [14]. The whole plots were then harvested with a silage harvester, most of the aboveground biomass being exported.

### *Soil water content and mineral nitrogen*

Soil nitrate and ammonium contents were measured along with soil water content (SWC) each year in early November (mid-autumn) and mid-March (late winter). Soil cores were collected down to a depth of 150 cm with a hydraulic coring device. Soil cores were taken from 2006 to 2014 in all treatments, except in autumn for late harvested perennials (because the plots were not accessible to the coring device) and in autumn 2006 for all perennials.

The cores were divided into five layers (0-30, 30-60, 60-90, 90-120 and 120-150 cm). Composite soil samples were formed by mixing six soil cores in each plot. The samples were frozen immediately after collection until extraction and subsequent analysis. After thawing, gravimetric water content was determined and nitrate and ammonium were extracted using a KCl solution (1 M) and analysed by continuous flow colorimetry. Soil water content (mm) and soil nitrate and ammonium (kg N ha$^{-1}$) were calculated by taking account of the bulk density which was measured for each layer with steel cylinders [23].

*Nitrate concentration in subsoil*

In order to evaluate nitrate leaching in the subsoil, soil cores were taken down to 390 cm on mid-November 2011 using a mechanical soil corer. One soil core (80 mm diameter) was collected in each plot with a N+ treatment. It was divided into 13 layers of 30 cm thickness. For each layer, gravimetric water content and nitrate content were determined using the same method as above. Nitrate concentration in the soil solution (mg $NO_3$ l$^{-1}$) was calculated as the ratio of nitrate-N content (mg N kg$^{-1}$ soil) to soil water content (l kg$^{-1}$ soil) multiplied by 62/14.

*Assessment of drainage and nitrate concentration in drained water*

The SWC and soil nitrate content measured in early November were used to initialize the STICS model which allowed assessment of drainage and nitrate leaching below 150 cm during winter. STICS is a dynamic model which simulates the carbon, water and N balances of the soil-crop system with a daily time step by taking into account the impact of weather, soil, crop and management practices [25]. Water transfer is simulated using a reservoir type model and nitrate movements using the “mixing cells” concept [26]. The model has been successfully evaluated for simulating water and N dynamics in various conditions including bare soils [27-29]. The software and documentation are freely available on the web site at http://www6.paca.inra.fr/stics_eng. We performed a total of 256 simulations (12 treatments × 3 blocks × 7 years). The first winter (2006-07) was not simulated because of the lack of initial data for perennial crops. All plots were simulated as bare soils (*i.e.* without plant) from the date of soil measurement in November to a crop-specific date in early spring (called *date tf*), assuming that crops had no significant growth during this period and influenced neither the water content nor the mineral N content in soil. *Date tf* was set as mid-March for established crops (fescue, alfalfa and triticale) which started their regrowth at this time, April 3 for miscanthus E and April 9 for switchgrass E (average dates of emergence of perennial crops), April 17 for resown fescue and alfalfa and May 20 for sorghum (average dates of sowing). We assumed that most of the annual drainage occurred during this simulation period given our pedo-climatic conditions and in agreement with Beaudoin et al. [30], although some late spring events in wet years might have slightly contributed to drainage. Input data for soil characteristics were estimated for each soil layer from direct measurements [23]. The water content at field capacity was taken as the median of all gravimetric measurements made in mid-March (from 2007 to 2014), except for the upper two layers (0-60 cm) in which water content could be significantly affected by evaporation: in these layers, years with a negative water balance during the ten days before sampling were excluded. For each plot and each winter, the model predicted the amounts of drained water and leached nitrate and the mean nitrate concentration of the drained water.

The volumetric soil water contents from 0 to 210 cm depth were monitored continuously in N+ treatments using water content reflectometers (Campbell Scientific CS616), as indicated previously [23]. The ability of STICS to simulate water fluxes was evaluated by comparing the simulated SWC (0-150 cm) to the values obtained with CS616 probes on a daily basis for the whole simulation period. Soil nitrate contents simulated and measured in mid-March were also compared in all plots using statistical criteria: root mean square error (RMSE) and mean deviation (MD). Although the results were satisfactory for SWC, STICS was found in a first run to overestimate the soil nitrate content in most situations, except after alfalfa destruction. This was attributed to an excessive simulation of net N mineralisation. We assumed that N immobilisation occurred due to crop residues (stubble, roots and rhizodeposits) left by the crops in November and decomposing during autumn and winter. Nitrogen immobilisation was simulated by a virtual addition on the first day of simulation of organic residues into the soil (0-30 cm) with a C:N ratio of 40 which might mimic root materials decomposing in the soil. The amount of virtual residues was fitted for each crop using a trial-error approach. In the case of alfalfa destruction in 2011 and 2013, the C:N ratio of the residues had to be decreased (set at 10) in order to simulate enhanced N mineralisation. A sensitivity analysis was performed to test the effect of varying the amount of residues on nitrate leaching and nitrate concentration in drained water.

We previously showed that all crops except sorghum had root extension below 150 cm and consumed water between 150 and 210 cm depth [23]. As a consequence, measurements made in early November down to 150 cm underestimated the total soil water deficit (SWD), *i.e.* the difference between SWC at field capacity and actual SWC, and the drainage simulated with STICS at 150 cm overestimated the water recharge towards the aquifer. The measurements made with CS616 probes allowed us to assess also the SWD between 150 and 210 cm in early November. Drainage below 210 cm was then calculated as the difference between drainage simulated by STICS at 150 cm and SWD [150-210]. Since only N+ treatments were monitored with these probes, we calculated the ratio between SWD [120-150] and SWD [150-210] and applied this ratio to the N- treatments, assuming that the distribution of water uptake within the soil profile was equivalent in both treatments. We also assumed that nitrate concentration was identical at 150 and 210 cm. Nitrate leaching at 210 cm was then calculated annually using the corrected drainage at 210 cm and the simulated nitrate concentration at 150 cm.

### *Statistical analyses*

All statistical analyses were performed using R [31]. The influence of preceding crop, N rate, year and their interaction on drainage and nitrate concentration were evaluated by analysis of variance (ANOVA). ANOVA was also performed to assess the effects of rotation and N rate on mean SWC, soil nitrate and ammonium measured over seven years, mean drainage and nitrate concentration. The effects of rotation and N rate on nitrate concentration in soil solution measured in autumn 2011 were evaluated for each soil layer using a third ANOVA.

Two linear mixed-effect models were used: the first one adapted to a split-block design (with blocks, rotation × blocks and nitrogen × blocks interactions as random factors) was used for perennial crops and the second, adapted to a split-plot design (with blocks and rotation × blocks interaction as random factors), was used for the other crops. The *lme* function of the *nlme* package was used for model fitting [32]. Significant differences ($p<0.05$) between treatments were detected using the *lsmeans* function [33]. The assumptions of ANOVA were checked by visually examining the residuals against predicted values and using the Shapiro-Wilk and Levene's tests. Logarithm or Box-Cox transformation was used if necessary to satisfy these assumptions.

## Results

### *Soil water and mineral N contents in early November and mid-March*

Soil water, nitrate and ammonium contents measured each year in early November and mid-March are given in Online Resource 4. The three variables differed significantly between treatments and years and significant rotation × year interactions were also observed.

The mean SWC (0-150 cm) measured in early November over seven years (2007-2013) varied significantly between rotations and N rates for perennial crops (Table 2). The highest value for perennial crops was observed for Swi E N- (476 mm) and the lowest for Mis E N+ (409 mm). Soil water content was higher under unfertilized than fertilized perennial crops and higher under annual crops than semi-perennial crops (461 *vs.* 410 mm). The mean SWC measured in mid-March over seven years (2008-2014) was higher than SWC in early November and much less variable between treatments. A significant rotation × nitrogen interaction was found for perennial crops but differences between treatments were small: SWC ranged from 515 to 525 mm. The mean value for semi-perennial and annual crops was 505 mm. Observed values in mid-March were close to the estimated SWC at field capacity: 527 mm for perennial and 516 mm for the other crops. The slight difference between the two groups was attributed to differences in soil texture rather than a crop effect, since the west part of the experiment (perennial crops) had a slightly finer texture than the east part (other crops) with 228 *vs.* 213 g $kg^{-1}$ of clay content over 0-150 cm. The variability of SWC among years was much higher in early November than in mid-March: 390 to 490 mm in early November *vs.* 501 to 527 mm in mid-March on average for all treatments.

The mean soil nitrate content measured in early November over seven years was dependent on rotation for semi-perennial and annual crops and on rotation × nitrogen interaction for perennial crops (Table 2). For perennial crops, it ranged from 11 (Swi E N-) to 35 kg N ha$^{-1}$ (Mis E N+). For the other crops, it was lower in Fes-Alf (26 kg N ha$^{-1}$) than in the other rotations (37 kg N ha$^{-1}$ on average). The mean soil nitrate content in mid-March did not differ between Mis E and Mis L and was slightly lower in Swi E than in Swi L. It was higher in Alf-Fes (57 kg N ha$^{-1}$) than in the other rotations. In early November, more than half of the total soil nitrate content (0-150 cm) was located in the first layer and 84% was found in the two upper layers (0-60 cm). The soil nitrate was located slightly deeper in mid-March (44% and 73% in the 0-30 and 0-60 cm layer respectively). Regarding variability among years, soil nitrate in early November was rather stable between 2007 and 2013 (the average of all treatments varying from 21 to 35 kg N ha$^{-1}$) whereas it markedly increased in mid-March in 2011 and 2014 after alfalfa destruction. The soil nitrate content was also much higher at the beginning of the experiment in April 2006, with 183 and 133 kg N ha$^{-1}$ for perennial and other crops respectively, but decreased markedly during the first two years.

The mean soil ammonium content measured over seven years was generally low: 13 and 15 kg N ha$^{-1}$ in early November and mid-March respectively (Table 2). Soil ammonium in early November was very stable over time since the average of all treatments varied between 10 and 16 kg N ha$^{-1}$. In mid-March, it varied from 6 to 23 kg N ha$^{-1}$ depending of the year.

*Nitrate concentration in soil and subsoil in 2011*

Nitrate concentration in the soil solution was measured in N+ treatments down to 390 cm in November 2011, *i.e.* five years after the beginning of the experiment (Fig. 1). Nitrate concentration was high in the upper layer (0-30 cm) and decreased with depth until 150 cm for all treatments. There was no significant difference between treatments under perennial crops whatever the soil layer, and no significant difference below 180 cm for the other crops. Nitrate concentration down to 180 cm was higher in Sor-Tri and Alf-Fes (*i.e.* after sorghum and alfalfa) than in the other two rotations. The high concentration in the upper layer after sorghum is attributed to a reduced N uptake in 2011 associated with a low biomass production due to a dry weather after sowing which hampered the crop establishment. Nitrate concentration in soil water under perennial crops was very low between 60 and 300 cm (2 mg $NO_3$ $l^{-1}$ on average). It increased below and reached 25 mg $NO_3$ $l^{-1}$ on average in the 360-390 cm layer, with a large variability. Conversely, nitrate concentration under the other crops was fairly constant from 120 to 390 cm and averaged 8 mg $NO_3$ $l^{-1}$.

*Ability of STICS to reproduce soil water content evolution during winter and soil nitrate in mid-March*

Before analysing the drainage and leaching fluxes simulated by the STICS model, we checked the ability of the model to reproduce the observed evolution of SWC during winter and the measured soil nitrate contents in mid-March. The daily measurements of water content made with CS616 probes during winter showed that SWC increased after early November to reach the field capacity during winter and remained rather stable until early spring. STICS could predict this evolution without bias and with little dispersion since the mean difference was -4 mm and the mean RMSE was 14 mm, close to the standard deviation of measurements. The good agreement between observed and simulated SWC (Fig. 2a) validates the assumption that crops had a negligible transpiration during the winter period and indicates that the simulated water fluxes calculated by the model are reliable.

STICS was also able to reproduce the amount of soil nitrate measured in mid-March after calibration of the N mineralisation rate (Fig. 2b). The RMSE was 16 kg N $ha^{-1}$. The model slightly overestimated the soil nitrate content over 0-150 cm (MD = 7 kg N $ha^{-1}$), but not at depth since RMSE and MD in the lower layer (120-150 cm) were small (RMSE = 3 kg N $ha^{-1}$ and MD = 1 kg N $ha^{-1}$).

We performed a sensitivity analysis of the model to the amount of virtual residues added to mimic the N immobilisation in soil. A 50% increase (respectively decrease) in the amount of added residues increased the amount of soil nitrate content by 2% (respectively 19%) and the nitrate leaching by 1% (respectively 1%). A 100% decrease of the amount of virtual residues (no residues) increased net N mineralisation by 15 kg N $ha^{-1}$, soil nitrate in mid-March by 42% and nitrate leaching by 3% only. We conclude that nitrate leaching predicted by the model was almost unaffected by the tuning of net N mineralisation. Indeed, the mineralisation in the upper soil layer had a negligible contribution to the nitrate leaching due to the moderate rainfall during the drainage period. The optimised value of net N mineralization between early November and mid-March was small in all treatments (5 kg N $ha^{-1}$ on average) except after the destruction of semi-perennial crops. In these cases, N mineralization reached 25 kg N $ha^{-1}$ after fescue and 67 kg N $ha^{-1}$ after alfalfa destruction.

*Evolution of drainage and nitrate concentration in drained water*

Drainage, *i.e.* downward water flux below 210 cm, was influenced by preceding crop, year and their interaction (Table 3). It varied widely between crop types for a given year and between years for a given treatment (Table 4). Significant effects of nitrogen and nitrogen × year interaction were also found for perennial crops. Indeed, the amount of drained water under perennials was similar in the two nitrogen treatments until 2009-10 and became higher (significantly in five cases out of eight) in the unfertilized treatment during the following years. Drainage varied widely between years, from 41 mm in 2009-10 to 192 mm in 2013-14 (average across all treatments). It also varied with crop rotation, from 227 mm after triticale in 2013-14 (Tri-Sor rotation) to 0 after alfalfa in 2009-10 and 2010-11 (Fes-Alf rotation).

Nitrate concentration in drained water was less variable than drainage (Table 5) although it was dependent on preceding crop, year and their interaction (Table 3). N fertilization rate also influenced nitrate concentration under perennial crops and a significant interaction between the three factors (preceding crop, nitrogen and year) was found for the other crops. The highest value (98.2 mg $NO_3$ $l^{-1}$) was found after Mis E N+ in 2007-08 and the lowest (0.6 mg $NO_3$ $l^{-1}$) after fescue N- in 2012-13. Nitrate concentration under miscanthus E exhibited the highest temporal variability (Fig. 3). It reached 83.3 mg $NO_3$ $l^{-1}$ in 2007-08 (average of N- and N+), decreased to 12.4 mg $NO_3$ $l^{-1}$ in 2008-09 and remained below 10 mg $NO_3$ $l^{-1}$ during the following years. Nitrate concentration under switchgrass E was significantly lower during the first two years and much more stable over time, varying between 1.1 and 6.1 mg $NO_3$ $l^{-1}$ (average of N- and N+). Annual crops produced concentrations ranging from 4.5 to 15.3 mg $NO_3$ $l^{-1}$ (average of the two rotations, N- and N+). A small increase in nitrate concentration was observed during the transitions between alfalfa and fescue, *i.e.* in 2008-09, 2011-12 and 2013-14 (average 10.2 *vs.* 3.2 mg $NO_3$ $l^{-1}$ in the other years).

Nitrogen leaching calculated annually was low (data not shown). It varied from 0 (Fes-Alf in 2009-10 and 2010-11, no drainage) to 13.8 kg N $ha^{-1}$ (Mis E N+ in 2007-08).

*Comparison between observed and simulated nitrate concentrations in the subsoil*

Another evaluation of the STICS model regarding drainage and N leaching was made by comparing model predictions with the nitrate concentrations measured in the subsoil. STICS predicted the mean nitrate concentration of the drained water below 210 cm during four years (2007-2011). This concentration was compared with that measured in the subsoil below 210 cm in November 2011, in a layer containing the same amount of water as the cumulative drainage predicted by the model. Assuming that nitrate was conservative and that dispersion was much smaller than convection during the downwards transport of nitrate, the two concentrations should be similar. The results of this comparison are given in Table 6. They show that the observed and simulated concentrations were close in all treatments, except Mis E. Excluding this treatment, we obtained a good, unbiased relationship between the two estimates: the regression equation between simulated and observed values was $y = 1.03\ x$ ($R^2 = 0.71$; $n = 5$), validating the simulations made with the STICS model. The simulated concentration for Mis E was much higher than the observed one (41.9 *vs.* 0.4 mg $NO_3$ $l^{-1}$). The high simulated value is mainly due to the high losses simulated in 2007-08. This discrepancy suggests that the hypothesis of conservative transport is not valid for this treatment, and that most of the nitrate leached during this year was taken up later by the crop. Nitrate uptake at depth is consistent with the deep rooting system of miscanthus previously characterized on this site [23].

*Mean drainage, nitrate leaching and nitrate concentration over seven years*

The amounts of drained water and nitrate leached below 210 cm averaged over seven years are presented in Table 7, as well as the mean nitrate concentration in drained water. The amount of drained water ranged from 56 mm $yr^{-1}$ for Mis E N+ to 142 mm $yr^{-1}$ for Tri-Sor N-. The mean drainage was higher for annual (133 mm $yr^{-1}$) than for semi-perennial crops (64 mm $yr^{-1}$) and intermediate for perennial crops.

N leached calculated over seven years represented 2.0 kg N $ha^{-1}$ $yr^{-1}$ on average for all treatments. It was very low for Swi and Fes-Alf (< 1 kg N $ha^{-1}$ $yr^{-1}$), highest for Sor-Tri N+ (5.0 kg N $ha^{-1}$ $yr^{-1}$) and intermediate for the other treatments.

The mean weighted nitrate concentration varied between 2 and 23 mg $NO_3$ $l^{-1}$. It was influenced by crop type and N rate in the case of perennials. Nevertheless, the higher concentrations under Mis E were mainly linked to the year 2007-08 and probably overestimated due to subsequent nitrate uptake by the crop in subsoil. If we exclude this year, the mean nitrate concentration was 2.5 ± 0.5 mg $NO_3$ $l^{-1}$ for Mis E N- and 8.1 ± 4.1 mg $NO_3$ $l^{-1}$ for Mis E N+. The lowest concentrations under the other crops were found for the Fes-Alf rotation (average 3.4 mg $NO_3$ $l^{-1}$) and the highest for Sor-Tri and Alf-Fes (average 13.8 mg $NO_3$ $l^{-1}$).

### *Relationship between biomass production and drainage*

Biomass production of perennial crops ranged from 12.8 to 26.5 t DM $ha^{-1}$ $yr^{-1}$ over seven years. It was higher for miscanthus E than for switchgrass E and for N+ than for N- treatments. Biomass production of the other crops was lower: it ranged from 9.4 to 11.0 t DM $ha^{-1}$ $yr^{-1}$ for semi-perennials and from 9.4 to 12.1 t DM $ha^{-1}$ $yr^{-1}$ for annual crops. Fig. 4 represents the relationship between biomass production and water drainage in the different treatments over the seven-year period. Drainage under perennial and annual crops appeared to be strongly and negatively linked with biomass production: $y = -4.99\ x + 187$ ($R^2 = 0.99$; $p < 0.001$), drainage being lower with more productive treatments. It is likely that the lower drainage in fertilized perennial crops was due to a higher biomass production and therefore higher evapotranspiration. Semi-perennial crops had a different behaviour: they produced a lower drainage than annual crops, in spite of a similar harvested biomass.

### *Actual evapotranspiration and water use efficiency*

Assuming that water runoff was negligible (due to the very slight slope and moderate rainfall), we could calculate actual evapotranspiration (AET) between April 2007 and March 2014 by difference between precipitation + irrigation and drainage, since the SWC variation was negligible. The mean AET varied between 546 and 624 mm $yr^{-1}$ (for Tri-Sor N- and Mis E N+ respectively), with an inverse ranking to that of drainage. The water use efficiency (WUE), defined as the ratio between biomass production and annual AET, was much higher for miscanthus and switchgrass (3.9 and 2.7 g DM $l^{-1}$ respectively on average for N- and N+) than for the other crops (on average 1.6 and 1.9 g DM $l^{-1}$ for semi-perennial and annual crops respectively). The WUE of each species increased with biomass production and was higher in N+ than in N- (Fig. 5).

## Discussion

### *Water balance*

Large differences in drainage were found between crops and treatments, with values ranging from 56 to 142 mm yr$^{-1}$. The differences were mainly due to the disparities in SWC at the end of the growing season, *i.e.* in early November. The dates chosen to end the simulations in spring (*date tf*) had a minor effect on these differences because spring drainage was small, due to the rapid increase in evapotranspiration. The longest drainage period in spring occurred between triticale and sorghum but the mean drainage simulated between mid-March and the sowing of sorghum was only 19 mm, corresponding to 13% of the total drainage. Three crops were shown to have a maximum rooting depth deeper than 210 cm on this site [23]: miscanthus (300 cm), switchgrass (288 cm) and alfalfa (276 cm). Drainage might have been overestimated for these crops since water capture below 210 cm was not taken into account. However, we think that the SWD below 210 cm was small at the end of the growing season. Indeed, in November 2011, no significant difference in gravimetric water content was found between crops below 210 cm.

The mean drainage for Mis E N+ was close to that of semi-perennial crops and smaller than that of annual crops. This result may appear contradictory with earlier findings [23], showing that the maximal SWD occurring during the growing season was highest for semi-perennial crops and equivalent for perennial and annual crops. The higher SWD in deep soil layers under perennials was compensated by a lower SWD in the upper layer. Nevertheless, when the crops were compared at the end of the growing season, *i.e.* in early November, the SWD under perennial crops was close to semi-perennial crops and higher than for annual crops. This was observed both with gravimetric measurements and reflectometers. Therefore the difference in drainage between perennial and annual crops is likely to be due to a higher AET during the last part of the growing season, which is consistent with the differences in crop phenology and harvest dates.

Few studies have compared drainage or AET of several bioenergy crops at the same site. Using soil moisture measurements over four growing seasons in central Illinois, McIsaac et al. [15] estimated that AET from miscanthus was 104 mm $yr^{-1}$ greater than for a maize-soybean rotation, which is consistent with our results. They also found that AET was equivalent for unfertilised switchgrass and annual crops. At the same site, Hickman et al. [34] measured AET during one growing season with a residual energy balance approach. Evapotranspiration ranked in the following order: miscanthus > switchgrass > maize. Yimam et al. [35] compared the annual AET of switchgrass and sorghum in two sites in Oklahoma using a soil water balance approach. AET ranged from 493 to 546 mm $yr^{-1}$ and was greater for switchgrass than sorghum in two out of three site-years. In contrast, Abraha et al. [36] measured AET by eddy covariance during three years in Michigan and found similar values for switchgrass and maize (555 mm $yr^{-1}$ on average). In a study comparing annual and semi-perennial crops, Pugesgaard et al. [17] calculated that drainage under grass-clover was 61% of the drainage under winter wheat (191 *vs.* 312 mm $yr^{-1}$).

Our results confirm that perennial and semi-perennial crops often consume more water than annual crops, resulting in lower drainage. This trend was probably enhanced by the soil type, a deep soil with high available SWC. The inverse relationship between drainage and crop biomass indicates that the higher water consumption of perennial crops was linked to a higher biomass production. In fact, the WUE of the perennial C4 crops (harvested in October), calculated over a full year, was higher than that of other crops, the highest value reaching 4.3 ± 0.3 g DM $l^{-1}$ in the fertilized miscanthus. The comparison with literature data is difficult because of differences in climatic conditions between sites and in the period of calculation. Our values for miscanthus fell in the range of those obtained by Cosentino et al. [37] (2.6- 4.8 g DM $l^{-1}$) and by Triana et al. [38] (3.7-4.3 g DM $l^{-1}$) in Italy. The much higher values obtained by Beale et al. [39] in the UK (7.8-9.2 g DM $l^{-1}$) are not comparable because they were calculated on a short period of active growth (May-August). Zeri et al. [40] and Hamilton et al. [41] found a higher WUE for miscanthus than for switchgrass and annual crops (maize), in accordance with our results. The positive effect of N fertilisation on water use efficiency is also consistent with the review of Zwart and Bastiaanssen [42].

*N leaching and nitrate concentration*

The amount of nitrate leached and its average concentration in drained water were generally very low in our experiment. Over seven years, N leached was only 2.0 kg N $ha^{-1}$ $yr^{-1}$ on average for all treatments and nitrate concentration ranged between 2 and 23 mg $NO_3$ $l^{-1}$. These values are much lower than those usually observed in arable cropping systems under similar climates. In a small catchment area in northern France with conventional cropping systems based on winter wheat, sugar beet, spring pea, winter barley and winter rapeseed, Beaudoin et al. [30] reported mean N leaching of 27 kg N $ha^{-1}$ $yr^{-1}$ with a mean nitrate concentration of 49 mg $NO_3$ $l^{-1}$. Benoit et al. [43] compared the nitrate losses in conventional and organic cropping systems on commercial arable farms of the Seine Basin (northern France). N leaching represented 14-50 kg N $ha^{-1}$ $yr^{-1}$ in organic and 32-77 kg N $ha^{-1}$ $yr^{-1}$ in conventional farming, corresponding to a mean nitrate concentration of 53 and 106 mg $NO_3$ $l^{-1}$ respectively. In two long-term experiments also located in the Seine Basin, Constantin et al. [44] measured N leaching ranging from 13 to 36 kg N $ha^{-1}$ $yr^{-1}$ and nitrate concentration from 43 to 109 mg $NO_3$ $l^{-1}$.

An important factor explaining the low nitrate losses in our study is probably the soil type (deep loamy soil). Indeed, several authors have shown that nitrate leaching is affected by soil type, with lower losses in fine (*i.e.* clayey or loamy) than coarse-textured (*i.e.* sandy) soils [30,45,46,16]. Beaudoin et al. [30] found a negative relationship between nitrate concentration and soil water content at field capacity. They calculated that the mean nitrate concentration in deep loamy soils (120 cm depth, 423 mm at field capacity) was 31 mg $NO_3$ $l^{-1}$, *i.e.* closer to our results.

The small amount of N leached observed in annual crops could also be explained by crop types and management practices. The mean soil mineral nitrogen (nitrate + ammonium) content in early November for annual crops (45 kg N $ha^{-1}$ over 0-150 cm) was lower than that reported by Beaudoin et al. [30]: 57 kg N $ha^{-1}$ in deep loamy soils (0-120 cm). This could result from (i) the absence of grain legume in our crop rotation, since soil mineral nitrogen in autumn is enhanced after grain legumes such as pea [30,43]; (ii) the establishment of catch crops before the spring crop (sorghum) which has been shown to reduce soil mineral nitrogen in autumn and N leaching [44,30]; and (iii) the moderate N fertilisation even in N+.

Nitrate concentration under semi-perennial crops was in the same order of magnitude than that measured under alfalfa by Benoit et al. [43] or under grass-clover by Pugesgaard et al. [17] in shallower or coarser soils: 24 and 12 mg $NO_3$ $l^{-1}$ respectively. The introduction of alfalfa into arable cropping systems has been shown to reduce nitrate losses [43,47]. Our study showed that nitrate concentration increased after alfalfa or fescue destruction and ploughing, due to an extra N mineralisation in autumn and winter which was estimated at 65 kg N $ha^{-1}$ after alfalfa and 25 kg N $ha^{-1}$ after fescue. In spite of this flush, the nitrate concentration in this rotation remained quite satisfactory since the mean value of the four treatments with alfalfa and fescue was 9.0 mg $NO_3$ $l^{-1}$.

Nitrate losses and nitrate concentrations under perennial crops were most often smaller than under annual crops, except in winter 2007-08 which exhibited high losses under miscanthus. These losses were attributed to the high soil nitrate content at the beginning of the experiment in April 2006 and the slow crop growth during the first year: the aboveground biomass of miscanthus at the end of the first year was only 1.2 t DM $ha^{-1}$, limiting the N uptake by the crop. This effect of crop age was observed in several studies. Lesur et al. [16] assessed nitrate leaching during two winters in 38 commercial miscanthus fields. They found that crop age was the main factor influencing nitrate concentration which decreased from 31 mg $NO_3$ $l^{-1}$ after the first year to 7 and 3 mg $NO_3$ $l^{-1}$ after the second and third years respectively. High nitrate losses were also measured by Christian and Riche [21] following miscanthus establishment at Rothamsted (UK). The concentration dropped from 143 mg $NO_3$ $l^{-1}$ in the first winter to 13 and 9 mg $NO_3$ $l^{-1}$ in the second and third winters respectively in the unfertilised treatment. The high initial losses were probably favoured by previous crops (long-term grass removed four years earlier and winter pea as preceding crop) and by heavy winter rainfall. Smith et al. [20] also observed higher N losses during the first winter following crop establishment than in subsequent years both for miscanthus and switchgrass. During the second and third year, Christian and Riche [21], Smith et al. [20] and Lesur et al. [16] found nitrate concentrations similar to ours for unfertilised perennial crops. N leaching measured during three years under unfertilised miscanthus and switchgrass in Illinois by McIsaac et al. [15] was also close to our measurements (5 kg N $ha^{-1}$ $yr^{-1}$ on average for both crops) and much smaller than for a maize-soybean rotation. This low N leaching for unfertilised miscanthus crops was confirmed by Davis et al. [22] who found 2.6 kg N $ha^{-1}$ on average over 6 sites in the US during the fifth year after establishment. In light of these results, it appears that the establishment phase of perennial crops presents a greater risk of nitrate losses than the mature phase. However, in deep soils such as in our study, nitrate lost during the first years and present in the subsoil can be taken up later by the crops thanks to their deep rooting depth [23]. This probably happened in our experiment for miscanthus as shown by the deep cores taken in subsoil in 2011.

There is apparently no consensus on the effect of N fertilisation on nitrate leaching under miscanthus: in our experiment, fertilisation slightly increased nitrate concentration whereas much larger losses in miscanthus fertilised with 120 kg N ha$^{-1}$ and harvested late were reported by Christian and Riche [21] (30 kg N ha$^{-1}$ in third year) and Davis et al. [22] (17 kg N ha$^{-1}$ in fifth year). These losses may be nevertheless overestimated due to the shallow depth of measurements (90 and 50 cm respectively) which was above the effective rooting depth of miscanthus in these sites. Nitrogen leaching in the late harvest treatment was not quantified in our study. It is expected to be close to the early harvest because soil nitrate content in mid-March was similar in early and late harvest treatments, as well as nitrate concentrations in subsoil in 2011.

## Conclusions

This study indicates that perennial bioenergy crops when managed properly can be effective to reduce nitrate losses compared to conventional crops. Semi-perennial C3 crops compared to annual crops produced a similar biomass but consumed more water and reduced drainage, being more suitable in wet climates. The C4 perennial crops (miscanthus and switchgrass) also reduced drainage but were more productive. Since drainage was highly correlated to their biomass production, a trade-off has to be found between high biomass production and sufficient groundwater recharge. C4 perennial crops had the highest water use efficiency, which confirms their interest for water resource management. Water quality with respect to nitrate was favourable for all bioenergy crops tested. However, the establishment phase of miscanthus is a risky situation which has to be anticipated by lowering the amount of mineral nitrogen available at crop establishment by avoiding legumes or grassland as previous crops, growing a catch crop during the previous autumn, etc. These results were obtained in a deep soil under temperate conditions. Other studies suggest that the efficiency of perennial crops to reduce nitrate losses could be greater in shallow soils compared to conventional arable crops. The differences in drainage between crops could also be lower in these soils, but the biomass production is likely to be lower too. Further studies using experimental networks and/or soil-crop modelling are needed to explore these effects of soil (and climate) variability and help choosing the best locations for these crops.

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## Figures

**Fig. 1** Nitrate concentration in soil solution versus depth measured in November 2011 in N+ treatments of perennial crops (a) and other crops (b). Bars represent the standard deviations between blocks.

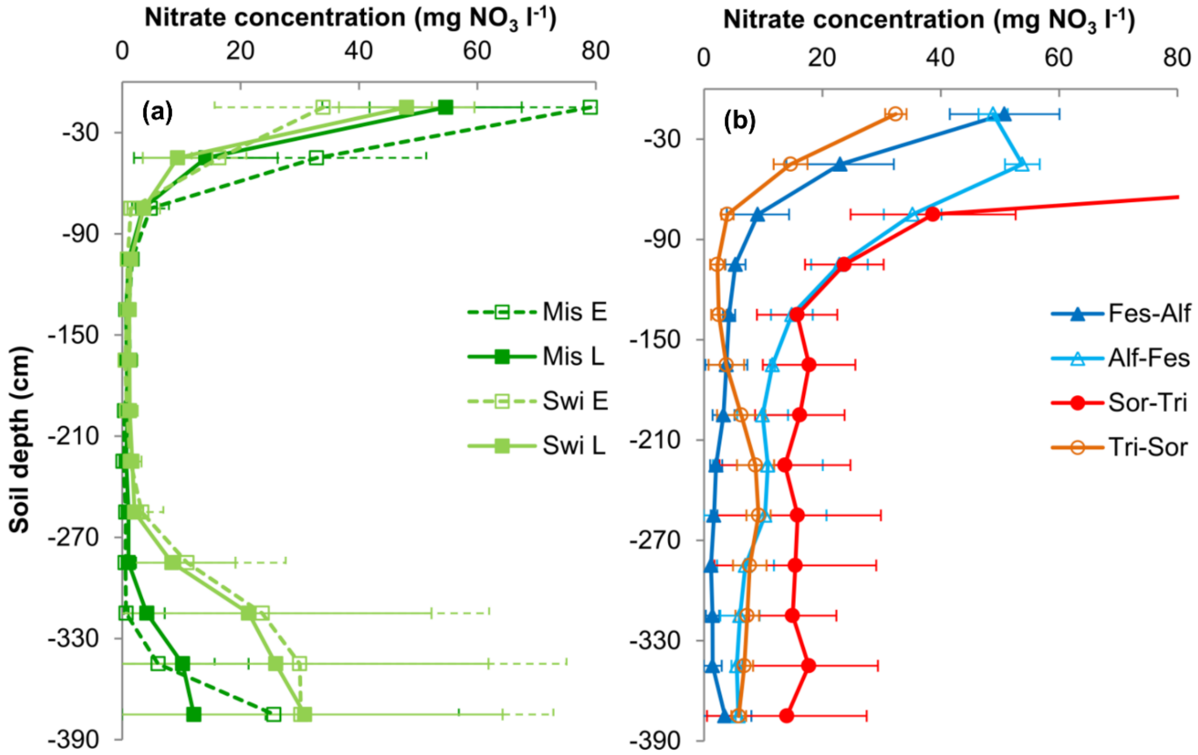


**Fig. 2** Comparison between observed and simulated values with STICS: (a) soil water content over 0-150 cm for each day of simulation in N+ treatments (each point represents one plot for a given day); (b) soil nitrate content over 0-150 cm in mid-March (each point represents one plot for a given year). The dashed lines are the 1:1 lines.

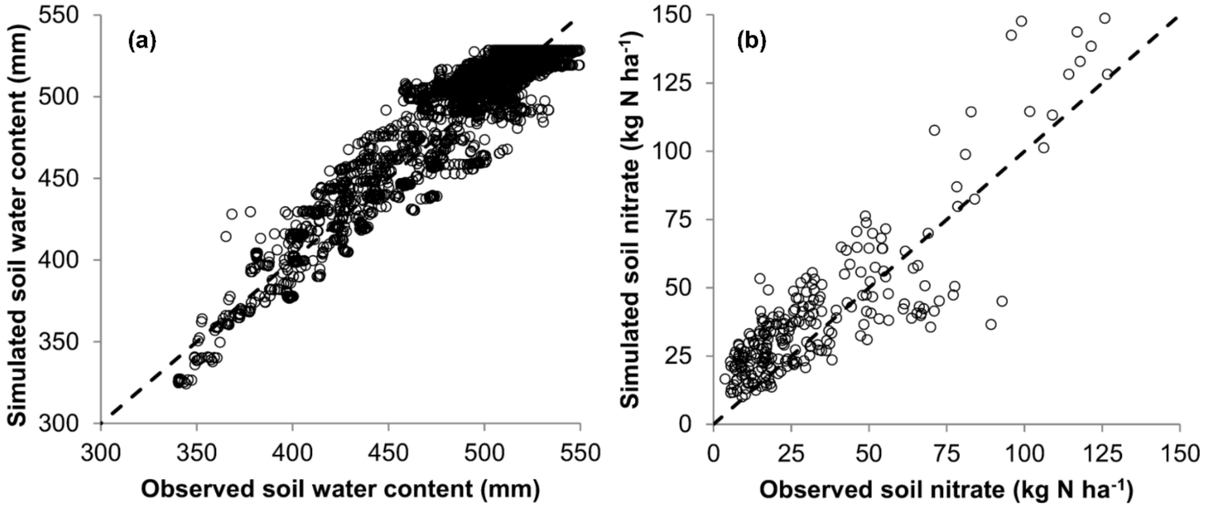
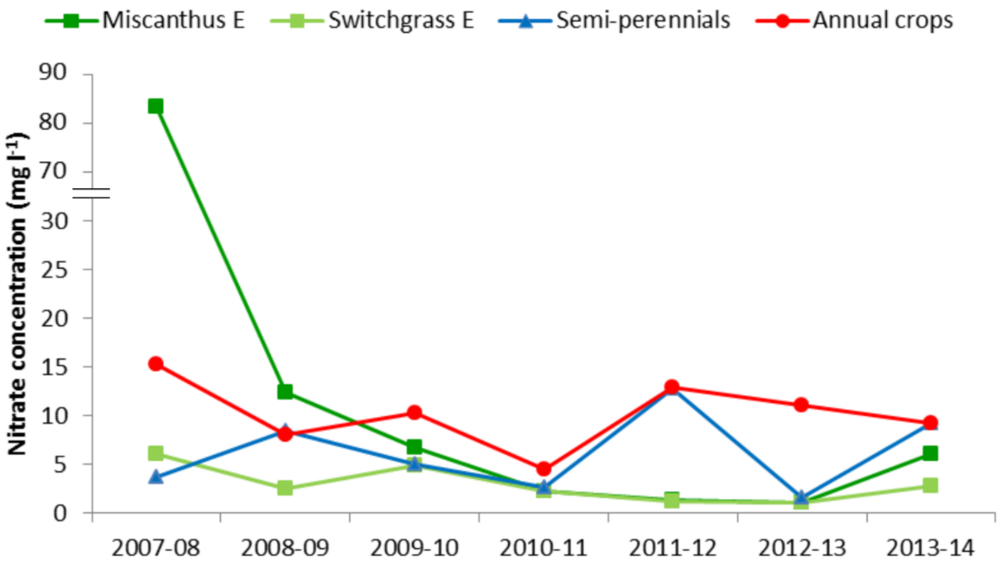


**Fig. 3** Evolution of the mean nitrate concentration in drained water (mg $NO_3$ $l^{-1}$) for miscanthus E, switchgrass E, semi-perennial (mean of Fes-Alf and Alf-Fes) and annual crops (mean of Sor-Tri and Tri-Sor). Data are averaged between N- and N+ treatments.



714 **Fig. 4** Relationship between drained water and biomass production (mean values, average of seven

715 years). Bars represent the standard deviations between blocks. The black line is the linear regression

716 without semi-perennial crops.

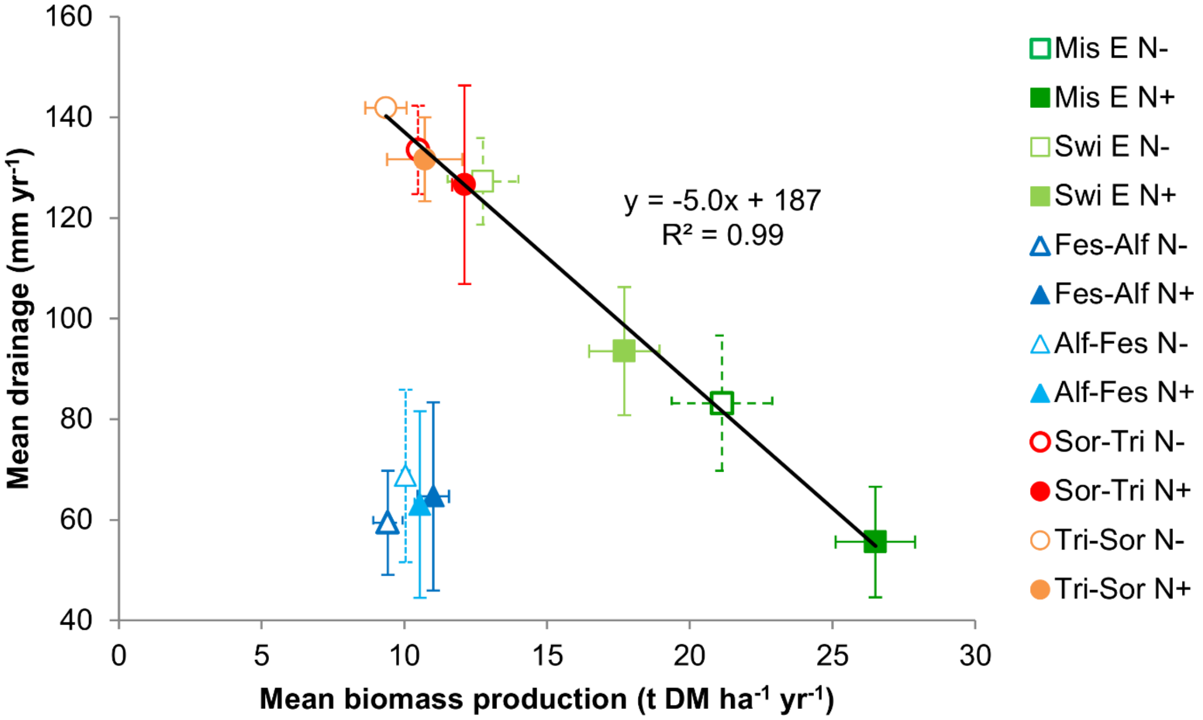


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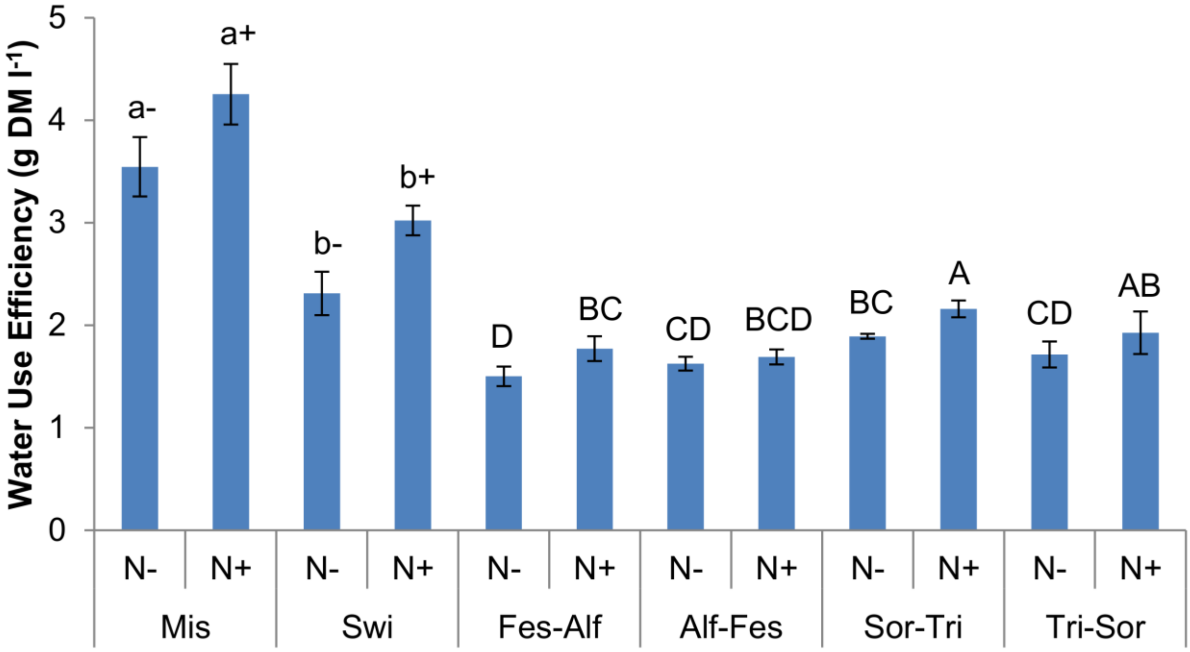
**Fig. 5** Water use efficiency in the various crop rotations (mean value over seven years). Bars represent the standard deviations between blocks. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation (without interaction with rotations).



## Tables

**Table 1** Treatments of the “Biomass & Environment” long-term experiment combining rotation and fertiliser-N rate: Mis = miscanthus, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fibre sorghum, Tri = triticale, CC = catch crop, E = early harvest (October), L = late harvest (February), N- = low fertiliser rate, N+ = high fertiliser rate. For triticale, the year corresponds to the harvest.

| Rotation | N rate | Crop and fertiliser-N rate (kg ha$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
| Mis E | | Mis | Mis E | Mis E | Mis E | Mis E | Mis E | Mis E | Mis E | Mis E |
| | N- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| Mis L | | Mis | Mis L | Mis L | Mis L | Mis L | Mis L | Mis L | Mis L | Mis L |
| | N- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| Swi E | | Swi | Swi E | Swi E | Swi E | Swi E | Swi E | Swi E | Swi E | Swi E |
| | N- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| Swi L | | Swi | Swi L | Swi L | Swi L | Swi L | Swi L | Swi L | Swi L | Swi L |
| | N- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| Fes-Alf | | CC/Fes | Fes | Fes | Alf | Alf | Fes | Fes | Fes | Alf |
| | N- | 0 | 120 | 80 | 0 | 0 | 0 | 120 | 120 | 0 |
| | N+ | 0 | 240 | 160 | 0 | 0 | 0 | 240 | 240 | 0 |
| Alf-Fes | | Alf | Alf | Alf | Fes | Fes | Alf | Alf | Alf | Fes |
| | N- | 0 | 0 | 0 | 40 | 120 | 0 | 0 | 0 | 0 |
| | N+ | 0 | 0 | 0 | 80 | 240 | 0 | 0 | 0 | 40 |
| Sor-Tri | | CC | Sor | Tri/CC | Sor | Tri/CC | Sor | Tri/CC | Sor | Tri/CC |
| | N- | 0 | 0 | 60 | 0 | 60 | 0 | 60 | 0 | 60 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| Tri-Sor | | Sor | Tri/CC | Sor | Tri/CC | Sor | Tri/CC | Sor | Tri/CC | Maize* |
| | N- | 0 | 60 | 0 | 60 | 0 | 60 | 0 | 60 | 0 |
| | N+ | 0 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |

[*] Fibre sorghum was replaced by silage maize (*Zea mays* L.) in 2014.

**Table 2** Soil water, nitrate and ammonium contents over 0-150 cm measured in early November (average of seven years from 2007 to 2013) and mid-March (average of seven years from 2008 to 2014).

| Rotation | N | Soil water content (mm) | | | | Soil nitrate (kg N ha$^{-1}$) | | | | Soil ammonium (kg N ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Early November | | Mid-March | | Early November | | Mid-March | | Early November | | Mid-March | |
| Mis E | N- | 434 (15) | b+ | 516 (2) | b | 20 (1) | b | 16 (2) | d | 10 (1) | a- | 13 (2) | b- |
| | N+ | 409 (4) | b- | 515 (1) | b | 35 (1) | a | 33 (2) | a | 15 (1) | a+ | 17 (2) | b+ |
| Mis L | N- | | | 518 (6) | ab | | | 17 (1) | d | | | 19 (4) | a- |
| | N+ | | | 515 (2) | b | | | 33 (1) | a | | | 22 (5) | a+ |
| Swi E | N- | 476 (4) | a+ | 523 (4) | ab | 11 (1) | c | 11 (1) | e | 11 (2) | a- | 15 (3) | ab- |
| | N+ | 446 (7) | a- | 520 (2) | ab | 19 (1) | b | 21 (1) | c | 15 (3) | a+ | 18 (2) | ab+ |
| Swi L | N- | | | 525 (4) | a | | | 14 (2) | de | | | 18 (2) | ab- |
| | N+ | | | 518 (4) | ab | | | 28 (4) | b | | | 19 (1) | ab+ |
| Fes-Alf | N- | 402 (6) | C | 506 (13) | A | 27 (8) | C | 35 (7) | B | 18 (2) | A | 16 (2) | A |
| | N+ | 404 (4) | | 508 (11) | A | 25 (7) | | 35 (3) | | 17 (2) | AB | 16 (2) | |
| Alf-Fes | N- | 416 (15) | B | 503 (20) | A | 36 (2) | AB | 56 (4) | A | 15 (1) | BC | 13 (4) | A |
| | N+ | 417 (7) | | 508 (13) | A | 35 (1) | | 58 (0) | | 16 (3) | B | 13 (2) | |
| Sor-Tri | N- | 465 (5) | A | 504 (9) | A | 37 (5) | A | 28 (2) | B | 8 (1) | E | 9 (1) | B |
| | N+ | 455 (11) | | 499 (20) | A | 53 (9) | | 38 (3) | | 11 (1) | D | 10 (2) | |
| Tri-Sor | N- | 460 (5) | A | 504 (2) | A | 31 (8) | B | 38 (9) | B | 11 (1) | D | 9 (1) | B |
| | N+ | 465 (13) | | 511 (6) | A | 30 (4) | | 38 (6) | | 11 (1) | D | 9 (2) | |

Values in brackets are standard deviations between blocks. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation (without interaction with rotations).

**Table 3** Factors influencing annual drainage and nitrate concentration in drained water over seven years (2007-08 to 2013-14).

| Factor or interaction | | Drained water (mm) | | Nitrate concentration (mg l$^{-1}$) | |
|---|---|---|---|---|---|
| | | Perennials | Other crops | Perennials | Other crops |
| Preceding crop | 1 | *** | *** | *** | *** |
| Nitrogen rate | 2 | *** | NS | ** | NS |
| Year | 3 | *** | *** | *** | *** |
| | 1 x 2 | NS | NS | NS | NS |
| | 1 x 3 | *** | *** | *** | *** |
| | 2 x 3 | *** | NS | NS | NS |
| | 1 x 2 x 3 | NS | NS | NS | ** |

Asterisks indicate probability levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant.

**Table 4** Annual drained water (mm) calculated between 2007-08 and 2013-14.

| Rotation | N | 2007-08 | 2008-09 | 2009-10 | 2010-11 | 2011-12 | 2012-13 | 2013-14 |
|---|---|---|---|---|---|---|---|---|
| Mis E | N- | 71 (31) | 41 (5) | 38 (17) | 71 (13) | 84 (37) | 70 (8) | 208 (20) |
| | N+ | 62 (14) | 46 (14) | 24 (30) | 51 (13) | 9 (15) | 40 (10) | 157 (5) |
| Swi E | N- | 129 (16) | 134 (15) | 55 (14) | 138 (8) | 122 (18) | 106 (11) | 208 (16) |
| | N+ | 126 (24) | 118 (15) | 28 (8) | 64 (18) | 61 (13) | 70 (0) | 188 (23) |
| Fes-Alf | N- | 39 (18) | 123 (45) | 0 (0) | 0 (0) | 39 (7) | 43 (8) | 173 (3) |
| | N+ | 46 (36) | 104 (66) | 0 (0) | 0 (0) | 50 (22) | 56 (10) | 196 (9) |
| Alf-Fes | N- | 55 (28) | 98 (29) | 6 (10) | 11 (19) | 123 (21) | 29 (18) | 160 (21) |
| | N+ | 54 (17) | 102 (30) | 0 (0) | 14 (25) | 110 (38) | 20 (20) | 141 (21) |
| Sor-Tri | N- | 48 (19) | 186 (10) | 53 (0) | 114 (6) | 139 (20) | 185 (16) | 211 (18) |
| | N+ | 54 (14) | 186 (20) | 48 (22) | 88 (14) | 132 (19) | 168 (43) | 211 (23) |
| Tri-Sor | N- | 171 (21) | 127 (9) | 134 (5) | 115 (8) | 108 (13) | 107 (18) | 231 (1) |
| | N+ | 173 (14) | 123 (26) | 103 (15) | 109 (20) | 101 (12) | 91 (5) | 222 (9) |

Values in brackets are standard deviations between blocks.

**Table 5** Nitrate concentration (mg $NO_3$ $l^{-1}$) in drained water simulated between 2007-08 and 2013-14 (mean annual weighted concentration).

| Rotation | N | 2007-08 | 2008-09 | 2009-10 | 2010-11 | 2011-12 | 2012-13 | 2013-14 |
|---|---|---|---|---|---|---|---|---|
| Mis E | N- | 68.3 (26.9) | 4.6 (3.0) | 5.3 (1.2) | 2.5 (0.1) | 1.0 (0.4) | 1.1 (0.4) | 2.6 (0.3) |
| | N+ | 98.2 (7.4) | 20.3 (17.8) | 8.2 (2.9) | 2.1 (0.9) | 1.8 (0.8) | 1.1 (0.2) | 9.6 (5.3) |
| Swi E | N- | 2.5 (0.0) | 2.2 (0.4) | 4.1 (0.9) | 2.3 (1.0) | 1.1 (0.5) | 1.0 (0.2) | 1.8 (0.7) |
| | N+ | 9.6 (5.7) | 2.8 (0.5) | 5.6 (0.4) | 2.1 (0.6) | 1.3 (0.8) | 1.2 (0.4) | 3.7 (0.2) |
| Fes-Alf | N- | 2.6 (0.1) | 2.8 (0.8) | 5.9 (0.1) | 3.0 (0.1) | 4.8 (1.1) | 0.6 (0.1) | 4.2 (1.4) |
| | N+ | 2.7 (0.1) | 2.5 (0.2) | 5.0 (0.5) | 2.9 (0.5) | 3.3 (0.8) | 0.7 (0.1) | 3.4 (0.4) |
| Alf-Fes | N- | 5.1 (2.0) | 6.9 (2.5) | 5.8 (2.9) | 2.2 (0.9) | 22.9 (9.9) | 1.9 (0.9) | 13.5 (8.4) |
| | N+ | 4.2 (1.3) | 21.8 (21.8) | 3.6 (0.1) | 2.6 (0.4) | 20.2 (2.4) | 3.0 (2.2) | 15.6 (7.4) |
| Sor-Tri | N- | 4.8 (1.8) | 6.7 (1.2) | 8.0 (4.8) | 5.0 (0.3) | 17.7 (6.8) | 14.2 (5.4) | 6.7 (0.5) |
| | N+ | 45.2 (27.6) | 10.1 (6.6) | 8.3 (5.9) | 5.5 (3.1) | 27.5 (10.4) | 13.2 (11.0) | 15.9 (10.3) |
| Tri-Sor | N- | 6.9 (4.9) | 9.1 (3.0) | 9.0 (2.3) | 3.8 (1.0) | 3.5 (1.1) | 8.4 (3.3) | 10.1 (0.9) |
| | N+ | 4.4 (1.0) | 6.2 (2.0) | 15.6 (9.9) | 3.7 (0.9) | 2.7 (0.7) | 8.6 (8.7) | 4.5 (2.8) |

Values in brackets are standard deviations between blocks.

**Table 6** Comparison between observed and simulated nitrate concentrations in the subsoil in 2011 in the N+ treatments. The simulated concentration is the mean nitrate concentration in drained water simulated over four years (2007-08 to 2010-11) using the STICS model. The observed concentration is the mean concentration measured in November 2011 in the subsoil layer containing the amount of water equivalent to the cumulative drained water during the four previous years.

| Rotation | N | Subsoil layer (cm) | Water stock (mm) | Cumulative drained water 2007-2011 (mm) | Nitrate concentration (mg $NO_3$ $l^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | | | Observed | Simulated |
| Mis E | N+ | 210-270 | 182 | 183 | 0.4 (0.3) | 41.9 (12.8) |
| Swi E | N+ | 210-320 | 336 | 336 | 8.2 (12.0) | 5.5 (2.6) |
| Fes-Alf | N+ | 210-260 | 157 | 151 | 1.9 (0.8) | 2.6 (0.1) |
| Alf-Fes | N+ | 210-260 | 160 | 170 | 10.6 (9.7) | 16.0 (15.2) |
| Sor-Tri | N+ | 210-330 | 366 | 376 | 15.0 (11.6) | 14.1 (9.2) |
| Tri-Sor | N+ | 210-370 | 494 | 507 | 7.8 (1.3) | 7.0 (2.6) |

Values in brackets are standard deviations between blocks.

**Table 7** Amounts of drained water, nitrate leached and nitrate concentration in drained water below 210 cm (mean values over seven years).

| Rotation | N | Drained water (mm yr$^{-1}$) | | Nitrate leached (kg N ha$^{-1}$ yr$^{-1}$) | | Nitrate concentration (mg NO$_3$ l$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| Mis E | N- | 83 (13) | b+ | 2.0 (0.9) | a- | 10.4 (3.5) | a- |
| | N+ | 56 (11) | b- | 2.8 (0.2) | a+ | 23.0 (3.6) | a+ |
| Swi E | N- | 127 (9) | a+ | 0.6 (0) | b- | 2.0 (0.0) | b- |
| | N+ | 94 (13) | a- | 0.8 (0.2) | b+ | 4.1 (1.4) | b+ |
| Fes-Alf | N- | 59 (10) | B | 0.5 (0.1) | C | 3.8 (0.5) | C |
| | N+ | 65 (19) | | 0.5 (0.2) | | 3.1 (0.6) | |
| Alf-Fes | N- | 69 (17) | B | 2.0 (1) | AB | 12.4 (4.1) | A |
| | N+ | 63 (19) | | 2.3 (0.1) | | 16.9 (5.3) | |
| Sor-Tri | N- | 134 (9) | A | 2.9 (0.9) | A | 9.6 (2.3) | A |
| | N+ | 127 (20) | | 5.0 (3.6) | | 16.4 (9.2) | |
| Tri-Sor | N- | 142 (2) | A | 2.4 (0.6) | B | 7.6 (1.9) | B |
| | N+ | 132 (8) | | 1.8 (0.7) | | 6.1 (3.0) | |

Values in brackets are standard deviations between blocks. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation.