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Nitrogen Budget of Short Rotation Forests Amended with Digestate in Highly Permeable Soils

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Abstract: Bioenergy crops are a promising option for integrating fossil fuels and achieving European environmental targets. Among these, Short Rotation Forestry (SRF) crops and biogas plants have been considered an opportunity for sustainable agricultural development due to their environmental benefits. In this case study, an N balance was performed by comparing an SRF *Platanus hispanica* plantation with a permanent meadow, both located in an area with highly permeable soils, using two different amounts of organic fertilization (digestate) for each system (0, 170 and 340 kg-N ha^{-1} y^{-1}). The results obtained indicate that, in the presence of highly permeable soils, the SRF is not effective in retaining N during the initial stage of growth, despite the use of a suitable application rate of digestate. Higher N leaching rates occurred in SRF crops compared to permanent meadows. Moreover, the N potential removal rate did not vary proportionally with the applied dose of digestate. To avoid N leaching excess, the annual applied N should be not only within 170 kg-N ha^{-1} y^{-1} (Nitrate Directive legal limits for nitrate vulnerable zone) but should also follow precise and accurate distribution practices, like: controlled grassing between the tree rows and soil's minimum tillage immediately after the digestate spreading.

Keywords: short rotation forestry; organic fertilization; N leaching; N emissions; N balance

1. Introduction

Resources other than fossil energy are required to face increasing problems such as the competition for energy resources and global climate change. One of the challenges of the bio-economy is to move from the use of fossil raw materials to sustainable production and utilization of renewable biomass. For instance, production of biogas using organic waste, as manure, represents a valuable resource, and, compared to fossil fuel utilization, the use of biogas yields a reduced carbon footprint [1,2]. Another good example is the use of woody biomass as renewable energy source to satisfy energy demands [3]. The European Commission (EC) prescribes an increase in renewable energy generation (at least 27% for 2030), of which biomass currently contributes to 63%. Among woody biomasses, Short Rotation Forestry (SRF) crops have good opportunities to be considered by the EC as a conventional agricultural product useful for agricultural diversification [4].

Biogas plant and woody biomass, besides replacing fossil fuels, can provide many environmental advantages. Biogas production through anaerobic digestion generates also digested slurry as organic waste, which is rich in nutrients [1]. The SRF crops are multifunctional systems that reduce wind and soil erosion, and favor carbon sequestration in the soil due to less intensive soil cultivation and accumulation of organic matter [5,6]. These benefits, in addition to energy production, are of great interest for cattle farming. However, if digestate can be considered a valuable and inexpensive fertilizer for SRF or meadow, the high concentration of livestock in areas with low crop coverage, has led to manure/digestate management concerns [7,8]—in particular, when it must be spread on highly permeable soil in NVZ (nitrate vulnerable zones). These areas have been designated at the risk of agricultural nitrate (NO_3^-) pollution by EC, which identified loading limits (170 kg-N ha^{-1} y^{-1}) for N fertilizers. Consequently, farmers have to follow mandatory rules to tackle NO_3^- loss from agriculture. In fact, the impact of organic and chemical fertilizers in soils and fresh and marine waters [9,10] is well known. Another key issue is the atmospheric pollution, mainly due to emissions of both ammonia (NH_3), which can lead to soil acidification [11] and nitrous oxide (N_2O), considered a major GHG (green-house gas) [7].

Although the economic impact of SRF crops has been investigated [12], comprehensive assessments of N dynamics when SRF crops are fertilized with digestate are rare, especially in highly permeable soils and during the first stage of SRF [13]. In fact, these environmental conditions could lead to high NO_3^- leaching rates due to the fast dynamic of soil hydrology and/or to high NH_3 volatilization rates due to a scarce soil cover. To support cattle and forage in permeable soils, there is a need for research to identify specific measures to efficiently recycle N and to assess plant responses to organic fertilization in a range of site conditions [14,15].

In the upper part of the Brenta River basin, a Forested Infiltration Area (FIA) [16], characterized by highly permeable soils (being in an upland alluvial fan), was cultivated with SRF and fertilized using digested slurry from the same farm.

In order to improve our knowledge on farms' sustainability in vulnerable areas, the main aim of this study is to assess if, and to what extent, SRF crops during their first stage can reduce the risk of N pollution caused by organic fertilization with digestate on permeable soils. In addition, the N balance was performed also in an adjacent traditional permanent meadow, in order to assess the sustainability of organic fertilization with digestate on SRF plantations with respect to grasslands. For this purpose, NH_3 volatilization, N_2O emissions and NO_3^- leaching, vegetation uptake and soil N content were investigated to gain a comprehensive N balance.

2. Materials and Methods

2.1. Experimental Site

The study area was located in northeastern Italy within a Quaternary unconfined aquifer composed by alluvial deposits of the Brenta River. The aquifer constitutes a large portion of the recharge area of the Venice lagoon drainage basin.

The SRF experimental site, due to the high soil permeability, is part of an experimental FIA [16]. The whole system was set during the spring of 2009 in a field formerly cultivated with maize and wheat rotation. The experimental site consists of a monoculture plot (60 × 50 m) of *Platanus hispanica* (Mill ex Muench, a hybrid of *Platanus orientalis* x *Platanus occidentalis*), which was planted with a distance of 2.5 m between rows and a distance of 2.0 m between columns (the ratio between the available distribution area and the total area is 2,5/7, thus 36%). Infiltration in the unsaturated zone is promoted by a system of ditches with a width of 1.0 m and spaced every 7.0 m (Figure 1).

An adjacent permanent meadow field, with the same soil characteristics, was chosen for the comparison with *Platanus hispanica*. In the upper 0.90 m, the soil is composed of a mixture of coarse and fine sediments (loamy gravel soil) characterized by fast infiltration rates. The soil is classified as Typic Hapludalfs loamy-skeletal, mixed, mesic soil according to the USDA classification. In the entire area, the presence of a significant content of fine sediments (silt and clay) in the top 45 cm decreases the soil's permeability and increases its water retention capacity. The groundwater level fluctuates approximately between 15 and 19 m below ground level (b.g.l.), thus the vadose zone is very thick and soil water logging conditions are not an issue at this site. The climate is subcontinental with about 1100 mm rainfall per year and mean temperature of 12.8 °C, a relative humidity of 76% and low average wind speed (6.5 km h^{-1}).

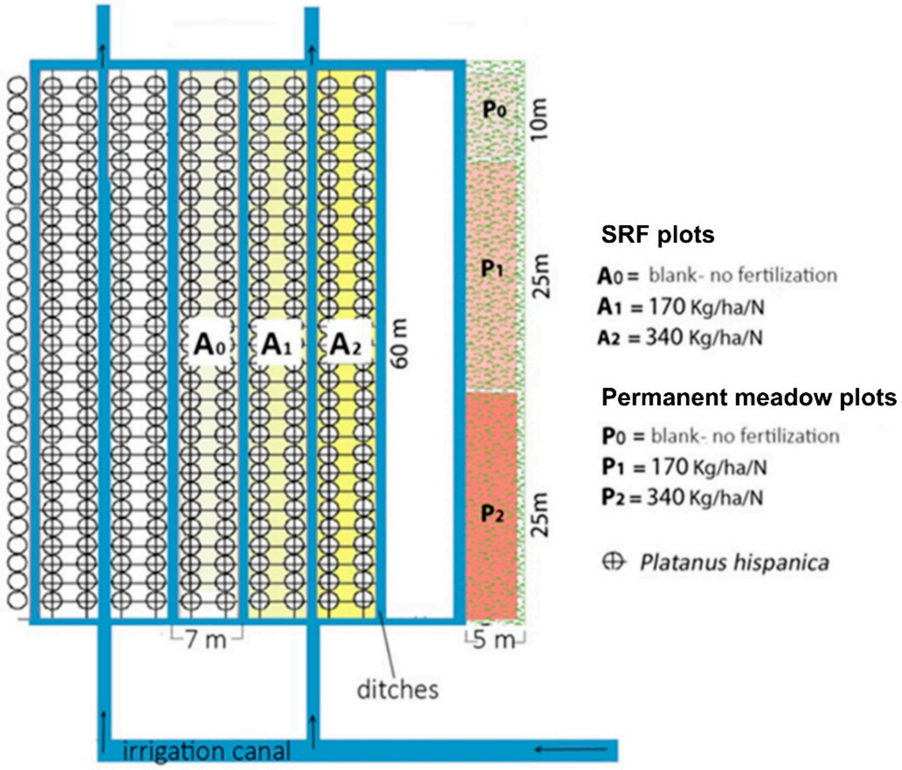

Figure 1. Experimental design: three plots (**A0, A1 and A2**) are set in the Forested Infiltration Area and three plots (**P0, P1 and P2**) are set in the permanent meadow. The legend shows the different amount of N added as digestate in each plot.

Measurements on N losses were performed over three experimental plots in the FIA (A_0–A_2 in Figure 1) and in the permanent meadow (P_0–P_2 in Figure 1), representing respectively "no distribution, "170 Kg-N ha^{-1} y^{-1}" and "340 Kg-N ha^{-1} y^{-1}", as shown in Figure 1. Unless otherwise specified, the experimental period was from January 2010 to September 2011, while the annual N balance was performed in the period between 10 May 2010 and 9 May 2011.

During the experiment, the two different amounts (170 and 340 kg-N ha^{-1} y^{-1}) of digested slurry were spread in three separate events, as reported in Table 1. The digestate was collected from a biogas plant treating dairy cow manure and ensiled energy crops (corn and sorghum silage). The digestate was applied with a 10 m^3 tank attached to a precision agriculture tractor delivering the planned amount of digestate (one third of the total in three times) using a flow meter (see Table 1). The spreading method employed a horizontal bar with 10 plastic tubes (inner diameter 5 cm) to allow an even distribution of digestate along the inter-rows in the FIA and in the permanent meadow, adopting the trailing shoe technique (low pressure distribution in a small groove 2 cm deep). Due to the presence of cobbles and to the high soil permeability, instead of burying the slurry in the soil, it was sprinkled in the surface, through a trailing shoe spreading system, slightly disturbing the superficial soil layer. In this way, the permanence on the soil's surface was short anyhow, but, at the same time, the first layer, rich in fine sediments, was fully exploited. In order to have comparable quantities of N for units of agricultural soil (kg ha^{-1}), in the SRF plots, the total amount of N (Table 1) was spread in the soil of the distribution area, which represents only the 36% of the total area (excluding the surface occupied by tree and ditches). The chemical and physical characteristics of the digestate were analyzed before each event (Table 2).

Table 1. Volume and N content from the digestate spreading during the experimental period.

	A1		A2		P1		P2	
Date	Volume (m^3 ha^{-1})	N (kg ha^{-1})	Volume (m^3 ha^{-1})	N (kg ha^{-1})	Volume (m^3 ha^{-1})	N (kg ha^{-1})	Volume (m^3 ha^{-1})	N (kg ha^{-1})
11 May 2010	15.2	45.8	23.8	71.4	18.8	56.3	37.5	112.5
29 June 2010	22.8	72	45.8	144	19	59.9	37.8	118.9
18 October 2010	21.4	45.4	46	97.6	19	40.3	37.8	80
Total annual	59.4	163.2	115.6	313	56.8	156.5	113.1	311.4

Table 2. Digestate characterization during the experimental period.

Date	DM (%)	VS (%TS)	Total N (mg L^{-1})	Total N-NH_4 (mg L^{-1})	pH (–)
11 May 2010	5.4	67.2	3000	2300	8.1
29 June 2010	5.2	64.6	3150	1700	8.4
18 October 2010	2.7	62.1	2120	1203	8.2

DM = dry matter, VS = volatile solids as % of total solids (TS).

2.2. Meteorological Parameters

The meteorological station of Rosà (VI), located 5 km from the field site, was used to monitor rainfall, wind speed, solar radiation, temperature and humidity. A rain gauge (WatchDog 3554WD1-Spectrum Technologies) was installed in the FIA site to record local daily rainfall. The regression coefficient of daily data between the rain gauge and the meteorological station (R^2 = 0.92) allowed for extending the data measured by the online weather station to the field site. The Penman–Monteith formula was applied to calculate the volumes of evapotranspiration, using the climatological data from the meteorological station [17].

2.3. Soil Hydrology

The soil water content, expressed as percentage of volumetric water, was recorded every 30 min through FDR Probes (Frequency Domain Reflectometry, spectrum SM 100) connected to a data logger (data-logging WatchDog 1000 Series Spectrum Technologies) and placed at different soil depths, at 15, 30, 60 and 90 cm b.g.l. The soil moisture system was placed both in the SRF and in the permanent meadow. The volumes of water infiltrated were estimated by applying the daily water balance method of Paniraghi and Panda [18]. It was assumed that run-off at this site is negligible [16] and that the errors in the assessment of actual daily evapotranspiration with the Penman–Monteith formula are small compared to the ones obtained using eddy correlation methods [19].

2.4. Soil Water Analyses

Soil water samples were collected in 26 sampling campaigns from May 2010 to August 2011 in three different points (replicates) for each plot at 30, 60 and 90 cm b.g.l. using tension lysimeters with a diameter of 63 mm and 60 unit of pressure (centibar). During dry periods, it was not always possible to maintain the pressure in all lysimeters, which caused the lack of some data for the 30 and 60 cm layers. Water samples were used to analyze the concentration of inorganic forms of N, and total dissolved N. Samples were filtered with 0.22 micron nylon filter disks.

The NO_3-N was measured by liquid chromatography. The N-NO_2 was measured by spectrophotometric analysis. The N-NH_4 was measured through spectrophotometry with the Nessler reagent. The total N was measured by spectrophotometric analysis after oxidation with a mixture of peroxydisulfate, boric acid and sodium hydroxide.

2.5. N Leaching

The daily balance of leached N was calculated by multiplying the daily volumes of deep seepage water below the root zone (90 cm) by the concentration measured during the sampling date considered most representative for that period, following the method of Ritchie [20].

2.6. Soil Analyses

Soil samples were collected using a manual drill at a depth of 0–30, 30–50, 50–80, 80–90 cm in three different points (replicates) for each plot. Samples were taken at the beginning and at the end of the experimental period. Each soil sample was analyzed for soil moisture, inorganic forms of N (NO_3-N, NO_2-N and NH_3-N) and total N. Soil moisture was determined gravimetrically after drying subsamples at 105 °C for 24 h. For soil extraction, 20 g were added in 200 mL of 2M KCL at 20 °C and kept stirring for one hour; then, 60 mL of the suspension was centrifuged for 10 min at 3000 rpm. Inorganic N forms were measured by spectrophotometric analysis following the method used for the water samples. The total N was determined by spectrophotometric analysis after the persulphate oxidation method.

2.7. Emissions of Gases (NH_3 and N_2O)

Emissions of NH_3 and N_2O from soil were measured after each digestate distribution. The measurement technique was based on closed chambers [21], a method that is suggested to be used when emissions come from experimental adjacent plots of limited extension. The chambers were cylindrical, with a volume of 0.005072 m^3, a height of 0.31 m and a surface area of 0.0109 m^2. The chambers were placed with open sides on the soil surface and internal air samples were taken every 2 min using polytetrafluoroethylene tubes connected to gas monitors adopting PAS technology (Photo Acoustic Spectroscopy, Bruel and Kjaer TM 1302, Nærum, Denmark). The chambers were left to stand on the soil from 12 to 20 min for each measurement (i.e., 6 to 10 gas samples for each measurement). NH_3 and N_2O fluxes from soil were calculated through the speed rate of gas concentration increase inside the chambers. Emission fluxes, recorded at different times after slurry spreading (time 0, immediately after spreading, after 1 h, after 3 h and after 24 h), were integrated over time in order to obtain the cumulative emission of NH_3 and N_2O, expressed as kg ha^{-1} d^{-1}. Measurements were stopped after 24 h, considering the time interval in which the majority of NH_3 is reasonably emitted [21–23].

2.8. N Deposition

The N_{tot} content in rain was measured by collecting water samples from a tank connected with a rain gauge. The amount of N deposition (N_r) was calculated by multiplying, for each period between two samplings, the total volumes registered for that period by the measured N_{tot} concentration.

2.9. N Contents of Vegetation Biomass

The assessment of woody biomass was performed on 26 July 2011, with sampling and weighing of young trees, adopting mainly dendrometric relationships. For shoots greater than or equal to 3 cm, a double entry table was used. This method was used also for shoots from 2 to 3 cm diameter at least 4 m high. This extension was considered adequate for a full chipping system. The formula used was:

$$Y = B_0 + B_1 X_2 H + B_2 H, \tag{1}$$

where: Y is the total weight (kg) of a single shoot, X is the diameter (cm) at 1.30 cm, H is the shoot height (m) and B_0, B_1, B_2 are the numerical coefficients of the regression, calculated as 5.9627, 0.0580 and 0.6224, respectively. The woody samples were dried and weighted, and their N content was assessed by the UNI CEN/TS 15104/2005 method.

In order to assess the whole vegetation biomass, three litter replicates of 1 m^2 for each plot were collected in different dates: (1) to measure litter biomass, leaves were collected throughout the fall period for the three SRF plots; (2) to measure herbaceous vegetation, samples were collected in August 2010 in all plots when the vegetation achieved its maximum production. Moreover, in the forested sites, where invasive weed (Amaranthus sp.) was present, it was completely collected, weighted and removed. All vegetation samples were dried at 50 °C for 24 h and weighted, and their N content was assessed by the UNI CEN/TS 15104/2005 method.

2.10. N Balance

The N balance was calculated in kg-N ha^{-1} over an entire year starting from the first digestate slurry spreading (10 May 2010), as the difference between the N entering (fertilizations and rains) and the N leaving the soil system (leaching, assimilation by woody and herbaceous biomass, emissions). The residual N (N_{res}) was obtained through the difference between measured input and output, as expressed and discussed below:

$$(N_f + N_r) - (N_L + N_{hb} + N_{wb} + N_e) = N_{res}, \quad (2)$$

where N_f stands for N input by fertilizer (digested slurry), N_r stands for N deposition by precipitation, N_L stands for N leaching at 90 cm b.g.l., N_{hb} stands for N removed through mowing of herbaceous biomass, N_{wb} stands for N assimilated in woody biomass, and N_e stands for emissions of NH_3 and N_2O after the slurry spreading events. N_{res} includes various N amounts not distinguishable between each other, as expressed below:

$$N_{res} = N_{den} + N_a + N_{err}, \quad (3)$$

where N_{den} stands for molecular N released in atmosphere by denitrification; N_{den} was not measured continuously, even if, during five experimental campaigns, soil samples at different depth (30, 60 and 90 cm) were taken in order to measure in situ denitrification (DNT) by the static core acetylene inhibition method [24]. N_a is equal to the N retained in soil and available for transformation and utilization (denitrification, leaching, uptake) during the last monitoring year. N_a only partially matches N_{tot} measured by soil analysis because N coming from inputs is not discernable from N already contained in the soil. The N_{err} is the sum of eventual and not measurable other N losses. It accounts for NH_3 losses occurring before manures wetted the soil surface, not measurable by chambers, N uptake by leaves, desynchronization of N dynamics caused by vegetation uptake and bacterial N_2 fixation [5].

The field dataset was analyzed via one-way analysis of variance tests (ANOVA), carried out in Microsoft Excel 2016 (Redmond, WA, USA) to investigate interrelationships between the vegetative treatments (forested infiltration areas and permanent meadows) with different levels of N fertilization levels from digestate. ANOVA tests determine whether there are differences in the means of two or more groups. These tests were used to investigate if there were any statistically significant differences in parameters collected in different soils. Statistical significance was defined by a threshold of * $p \leq 0.05$ for all statistic tests. Analyses of variance were performed on untransformed data and mean separation was accomplished using Tukey's honestly significant difference (HSD) test.

3. Results and Discussion

3.1. Components of the Soil N Budget

3.1.1. N Leaching

Figure 2 shows that the leaching of N-NO_3 in the soil profile in SRF is rather fast. The N-NO_3 concentration tends to disappear in the first 2–3 months after each digestate distribution. Moreover, the highly dynamic hydrology reduces significantly the soil buffering capacity as suggested by the N-NO_3 concentration from the surface down to 90 cm. The higher N-NO_3 concentrations recorded sometimes at 90 cm were due to the sampling period: as the residence time is strongly influenced by meteorological conditions, the high values of N-NO_3 recorded at the end of May 2010 were coupled with the heavy rainfall immediately after the first digestate slurry spreading. It is important to underline the unusual high baseline value in A0 (control plots) observed during the first three months of the experimental activities (Figure 2), which was probably due to the N residual from the previous agricultural activities (sweet corn cultivation). Data on N-NH_4 and N-NO_2 are not reported because they were negligible in concentration with respect to N-NO_3.

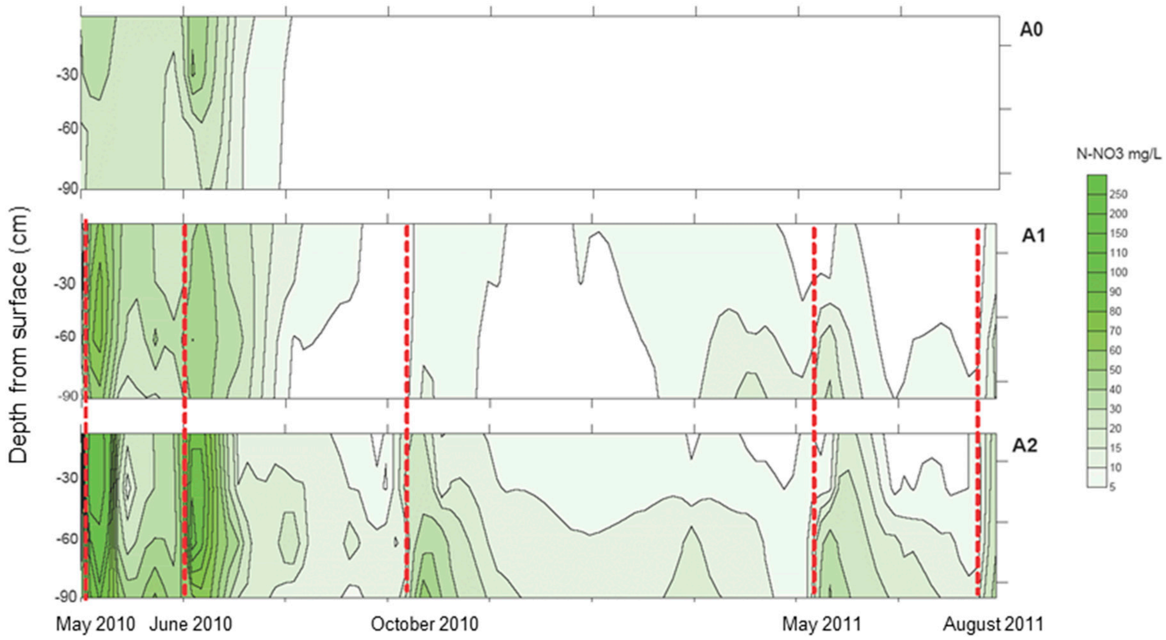

Figure 2. Contour plot of the N-NO_3 concentration in the 0–90 cm soil horizon obtained via Kriging interpolation method during the monitored period in SRF plots (**A0**–**A2**). The dashed red lines are the digestate spreading dates.

The only exceptions were some peaks of N-NH_4 (10–15 mg L^{-1}) observed at 30 cm in P1 and P2 plots, during 12 May 2010 and 26 October 2010 samplings, possibly associated with an incomplete nitrification of the N-NH_4 in the digestate distributed during the previous days. In terms of mass balance, the total amount of N leached from each plot is showed in Table 3.

Table 3. Percolation rate at 90 cm depth and leaching of N for each plot.

	Rainfall (mm)	Leaching at 90 cm (mm)	N-Tot Leaching (kg ha^{-1})
A0			35.2 ± 2.8
A1	1603	564 ± 113	81.4 ± 4.0
A2			111.2 ± 7.6
P0			14.8 ± 0.6
P1	1603	536 ± 107	34.9 ± 4.2
P2			82.9 ± 7.8

N leaching was significantly higher in the SRF plots than in the permanent meadow plots. This could be explained both considering a greater N uptake of permanent grass vegetation with respect to new tree plantations, and, by considering uneven spreading of N in the SRF soil, in fact the distribution area represents only 36% of the total field given that digestate was applied only in the inter-rows.

3.1.2. N Deposition

The annual rainfall between May 2010 and May 2011 was equal to 1603 mm; the average N content in rain was 2.1 mg L^{-1}. The N inputs from the rain in the permanent meadow amounted to 33.7 kg-N ha^{-1} y^{-1}, whereas, in the SRF plots, the net contribution that directly fell into the area of interest, excluding ditches, was 28.2 kg-N ha^{-1} y^{-1}.

3.1.3. N Emissions

The total N emissions (output) (Figure 3) were lower from SRF (3.46 and 4.60 kg-N ha^{-1} y^{-1}, respectively, for A1 and A2) than from permanent meadow (5.35 and 8.11 kg-N ha^{-1} y^{-1}, respectively, for P1 and P2), and the most were in the form of NH_3. N emissions from all the plots corresponded to 2.2–3.2% of the applied N (Table 1).

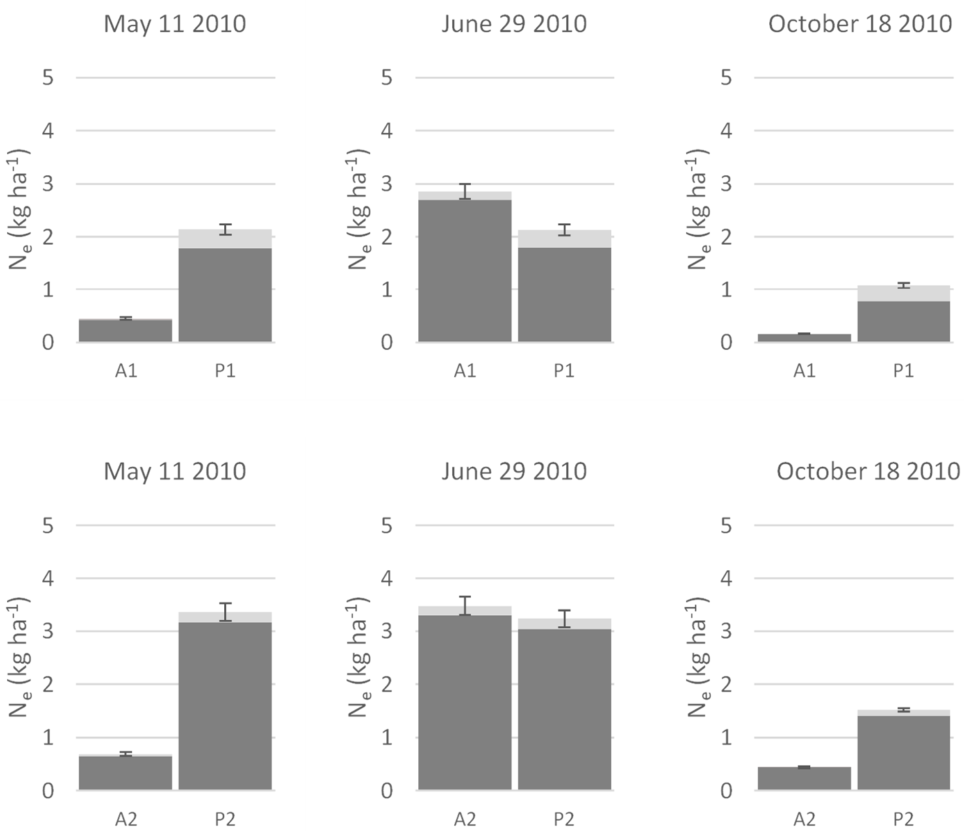

Figure 3. N emissions from A1, A2, P1 and P2 plots; in each column, dark gray is NH_3, light gray is N_2O (bars represent standard errors for each column). All the mean values are significantly different from each other (Tukey's HSD, * $p < 0.05$).

As confirmed by literature, NH_3 emissions from slurry applied in the field are quite variable, ranging from 0.0 to 60% of the applied NH_3 [25]. N_2O emissions ranged from 0.194 to 0.760 kg-N ha^{-1} y^{-1}, which corresponded to about 0.2% of the total N distributed, a value slightly lower than that reported in literature [22]. Moreover, weather conditions have a crucial importance in determining N emission [9]. The hot weather conditions that characterized 29 June 2010 led to higher NH_3 emission for A1 and A2 plots. This did not occur in P1 and P2 plots; in fact, the bare soil in May led to a higher emission with respect to late June, where weeds had a shadowing effect that may have reduced emissions. In October 2010, the low emissions were probably caused by a combination of spontaneous grass covering between rows, cool temperature and litter cover. The high variability in the presented data can also be related with the different chemical characteristics of the digestate (Figure 3), which have a great influence on NH_3 emissions [26].

3.1.4. Inorganic N in Soil

In the SRF plots, at the beginning of the experimental campaign, the soil was characterized by a high N content, probably because of the former N application for maize production. This is confirmed by the values observed in the control plots (A0). By comparing the initial (May 2010) and final (May 2011) phases, a reduction in N content in the soil was observed both in the SRF and in the permanent meadow plots. This gap is clearly higher in the permanent meadow plots, where high growth rates were observed (Table 4), thus the herbaceous vegetation uptake was the main process in N removal.

Table 4. Concentration of N in soil.

Depth (cm)	May 2010 (kg-N ha^{-1})						May 2011 (kg-N ha^{-1})					
	A0	**A1**	**A2**	**P0**	**P1**	**P2**	**A0**	**A1**	**A2**	**P0**	**P1**	**P2**
0–30	39.1	41.3	40.7	50.2	51.1	53.7	36.9	26.7	25.6	19.9	22.4	30
30–50	54.7	59.3	56.9	63.6	65.9	59.6	56.1	34.7	39.3	12.5	41.5	33.2
50–80	60.5	68.1	65.1	64.8	63.4	69.1	53.1	49.5	59.8	9.6	41.8	47.4
80–90	49.9	50.7	47.2	63.2	64.8	59.9	46.7	49.5	59.8	10.2	39.1	41.9
TOT	204.2	219.4	210	241.8	245.3	242.3	192.8	160.4	184.4	52.2	144.8	152.6

Depth(cm)	Difference * (kg-N ha^{-1})					
	A0	**A1**	**A2**	**P0**	**P1**	**P2**
0–30	−2.2	−14.6	−15.1	−30.3	−28.7	−23.7
30–50	1.4	−24.6	−17.6	−51.1	−24.4	−26.4
50–80	−7.4	−18.6	−5.3	−55.2	−21.6	−21.7
80–90	−3.2	−1.2	12.6	−53.0	−25.7	−18.0
TOT	−11.4	−59.0	−25.4	−189.6	−100.4	−89.8

* difference in N soil content between May 2010 and May 2011.

3.1.5. Nitrogen Content in Woody and Herbaceous Biomass

The total values of dry woody biomass estimated at the end of this experiment on 26 July 2011 were: 5.4, 6.4 and 8.3 t ha^{-1}, in A0, A1 and A2, respectively. The values show a low uptake of N by trees plantation at this stage: 19.9, 18.6 and 25.7 kg-N ha^{-1}, in A0, A1 and A2, respectively. Long-term studies have reported significant values of immobilized N in woody biomass [27], recorded values ranged from 110 to 300 kg-N ha^{-1} in a nine-year poplar plantation.

The values of total dry herbaceous biomass recorded from May 2010 to May 2011 in all the plots (Table 5) ranged from 6 to 13 t ha^{-1} y^{-1}. In the three SRF plots from 30 June 2010 to 23 August 2010, there was a significant growth of Amaranthus sp., an invasive herbaceous species.

Table 5. N amount in herbaceous biomass.

	Dry Herbaceous Biomass (t ha^{-1} y^{-1})	Available N (kg ha^{-1} y^{-1})	N Removed (kg ha^{-1} y^{-1})
A0	6	152	6
A1	9	218	24
A2	10	246	36
P0	8	0	188
P1	10	0	256
P2	13	0	340

The biomass values showed clear differences between the two control plots and the plots fertilized with digestate, and a clear and proportional effect of the fertilization was demonstrated. However, part or the whole N quantity has been removed (see Table 5). Unlike in the SRF system, where only Amaranthus sp. was removed, the above ground herbaceous vegetation in the permanent meadow was completely removed after mowing.

3.2. Nitrogen Balance

Nitrogen balance results are resumed in Table 6. In all the fertilized plots, for both SRF systems and permanent meadows, the N coming from digestate was the most important input, whereas, where fertilization was not carried out, only N from rain contributed. In SRF systems, the most important output was the N loss via leaching, estimated in 81 and 111 kg-N ha^{-1} y^{-1}, for A1 and A2, respectively. High levels of leaching could be related to both highly permeable soils and low levels of water uptake by the roots of the young trees. In fact, N retained by woody biomass was estimated at 19 and 26 kg-N ha^{-1} y^{-1}, respectively, for A1 and A2, whereas herbaceous weeds removed from the systems 24–36 kg-N ha^{-1} y^{-1}. Production of herbaceous biomass from the permanent meadow allowed the highest N export, which amounted to 256 and 340 kg-N ha^{-1} y^{-1}, respectively, for P1 and P2 thesis. Because of this, N leaching from the permanent meadow was lower than that from the SRF, amounting to 35 and 83 kg-N ha^{-1} y^{-1}, respectively for P1 and P2.

Table 6. Comparison of the N (kg ha^{-1} y^{-1}) balance among the different field plots. N_f = input by fertilization. N_r = input by rain. N_L = N leaching. N_{hb} = output by herbaceous biomass. N_{wb} = output by woody biomass. N_e = output by atmospheric emissions. N_{res} (not distinguishable) = N_{den} (denitrification) + N_a (available in soil) + N_{err} (not measurable N losses) all expressed in kg-N/ha-1/y-1. All the mean values are significantly different from each other (Tukey's HSD, $p < 0.05$).

	A0	A1	A2	P0	P1	P2
N_f	0.0	163	313	0.0	156	311
N_r	28	28	28	34	34	34
Total input	**28**	**191**	**341**	**34**	**190**	**345**
N_L	35	81	111	15	35	83
N_{hb}	6.0	24	36	188	256	340
N_{wb}	20	19	26	0.0	0.0	0.0
N_e	0.0	3.5	4.6	0.0	5.3	8.0
Total output	**61**	**127**	**177**	**203**	**296**	**431**
N_{den} + N_{err} + N_a	**−33**	**64**	**164**	**−169**	**−106**	**−86**

The highest levels of N_{res} (difference between N input and N output) were found in the SRF systems. In particular, the only positive values of N_{res} were found in A1 and A2 (64 and 163 kg-N ha^{-1} y^{-1}, respectively). Considering these data linked to the difference of N concentration in the soil (−59 and −26 kg-N ha^{-1} y^{-1}, Table 4), it was possible to suppose that N was not preserved into the soil, but, at least partially, was denitrified. As a matter of fact, some clues suggested that denitrification was an ongoing process: (i) the measured denitrification rates showed positive results in all the measuring campaigns, with an average rate of 1 ± 0.1 g N-NO_3 ha^{-1} d^{-1}; (ii) the N-NO_2 concentration in tension lysimeters was 0.42 mg L^{-1}, suggesting an active denitrification, with four isolated peaks over 1 mg L^{-1}, indicating that the limiting condition is the availability of the labile organic substrates, which usually leads to transient N-NO_2 accumulation [28,29]; (iii) the average redox potential measured in the tension lysimeters was −112 ± 7 mV, indicating anoxic-hypoxic conditions suitable for denitrification in soils [30]. All the above-mentioned clues can be considered as evidence of the ongoing denitrification process, although they cannot be used directly to quantify this process, which was then estimated via mass balance (Table 6). The high denitrification capacity could be linked to high amounts of distributed digestate in a smaller area, which can lead to anaerobic conditions in soils. In the permanent meadow, strongly negative values of N_{res} (ranging −86 to −169 kg-N ha^{-1} y^{-1}, passing from the highest level of fertilization to the lowest) and differences of N concentration in soils (ranging −89 to −190 kg-N ha^{-1} y^{-1}) suggested that soils became depleted of N, and that permanent meadows would benefit from higher levels of fertilization. This finding was further supported by the increase in production of herbaceous biomass when passing from P0 to P2.

4. Conclusions

The piedmont alluvial areas are very often the most vulnerable zones to nitrate, but, unfortunately, their vulnerability is not often considered. This study has shown that in highly permeable soil the N retention during the juvenile stage of SRF is quite scarce, and N is mainly leached through the soil and partially assimilated by weeds between tree rows. Instead, the permanent meadow showed high N uptake efficiency, leading to a reduction of N leaching in comparison with the juvenile SRF system, although more research is needed to quantify N leaching over longer time periods.

The study suggests that, for the juvenile stage of SRF, some measures for a sustainable use of digestate in SRF can be implemented, like: (i) a controlled grassing between the tree rows by removal of herbaceous biomass; (ii) a reduction of distributed amounts of digestate, at least for the first stage of the system; in particular, the available distribution area in the FIA being only 36% of the total surface (due to the presence of trees and ditches), it is suggested to reduce by the same proportion the amount of distributed digestate; and (iii) promote soil's minimum tillage practices immediately after the digestate spreading, in order to facilitate the slurry penetration into the soil. This will consequently limit the digestate's contact time with the atmosphere, thus reducing ammonia emissions. However, it should be considered that the latter operation could speed up N mineralization and leaching.

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