Nutr Cycl Agroecosyst (2010) 88:411–427
DOI 10.1007/s10705-010-9366-4

RESEARCH ARTICLE

The use of organic substrates with contrasting C/N ratio in the regulation of nitrogen use efficiency and losses in a potato agroecosystem

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Received: 9 July 2009 / Accepted: 4 April 2010 / Published online: 20 April 2010

Abstract The effect of the application of organic amendments of contrasting C/N ratio, combined with mineral N fertilizer, on the N budget of a potato crop was evaluated. The hypothesis was that the combination of substrates of contrasting chemical composition can improve the synchronization between N crop requirements and N availability in the soil, increasing yields and reducing losses to the environment. Potato plants were cultivated in microplots (0.225 m^2) in the tropical uplands of Venezuela, using mineral N (labeled with ^{15}N), poultry manure (C/N = 12), and rice hulls (C/N = 90) as N sources. Four treatments with the same total dose of N (25.0 g N m^{-2}) were applied: (1) MF: mineral fertilizer; (2) P + F: poultry manure and mineral fertilizer; (3) R + F: rice hulls and mineral fertilizer and (4) P + R + F: poultry manure, rice hulls and mineral fertilizer. Labeled and non-labeled N was measured in drained water and plant and soils compartments, and a N budget was established for each treatment. The ratio of N crop uptake to N losses was proposed as an ecological indicator of N use efficiency. The highest value (3.0) of this ratio was obtained in the treatment combining the two organic substrates of contrasting quality, P + R + F, followed by the R + F (2.0) and P + F (1.8) and the lowest ratio was obtained in the MF treatment (0.9). The P + R + F combination may represent a good soil amendment to obtain a high yield with a lower environmental impact, at least in the short-term. The generalization of these results to other soils and climates is discussed.

Keywords Andes · N budget · Nitrogen-15 · N use efficiency · Potato · Poultry manure · Rice hulls · Synchronization · Tropical mountains

Introduction

Significant amounts of the N added to agroecosystems by fertilization are lost by leaching, volatilization and denitrification leading to important economic and environmental impacts worldwide (Keeney 1982; Bohlool et al. 1992; IPCC 1992).

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Depending on fertilization (type, amount and schedule/timing), crop variety, management practices, and soil and climatic conditions, these losses can be as high as 50–80% of the added N (Frissel 1977; Bohlool et al. 1992; Powlson et al. 1992; MacDonald et al. 1997; Maidl et al. 2002; Chapin et al. 2002; Sheehy et al. 2005). Leached N can pollute water with nitrates leading to eutrophication and risks to human health, and gaseous losses of N_2O and NO contribute to the depletion of the ozone layer and to global warming (Grant 1994; Vermoesen et al. 1996; Ünlü et al. 1999; Halitligil et al. 2002). In addition to negative environmental impacts, N losses decrease farmers' income and agroecosystem efficiency, and raise energy consumption in agriculture. Consequently, one essential component for designing more sustainable agricultural systems is to improve the efficiency of crop N recovery and to reduce gaseous and leaching losses (Bélanger et al. 2003). To achieve these goals, N availability in the soil has to be matched to crop requirements in time and space, avoiding the accumulation of mineral N in the soil and therefore reducing the risk of losses. It has been hypothesized that temporal synchronization between N availability and crop requirements can be improved by combining the use of mineral fertilizers with organic amendments of different qualities and C/N ratios (Swift 1984, 1987; Myers et al. 1994). These combinations can improve the synchronization by regulating N mineralization and immobilization by microorganisms.

In the highest agricultural belt of the Venezuelan Andes, from 2,000 to 3,600 m altitude, potato constitutes the main commercial crop. The most widespread production system in the area is characterized by the application of high amounts of fertilizers, sprinkler irrigation, frequent application of biocides and the use of high-yield potato varieties. Nitrogen is applied combining different sources: mineral N (150–300 kg N ha^{-1}), poultry manure (10–30 Mg ha^{-1}) and rice hulls (30–90 Mg ha^{-1} in 2 year intervals) (Corpoandes 1995).

Due to the large amount of N applied, the combination of sources of contrasting quality, the light texture of the soil and the use of irrigation, potato crops in the Venezuelan Andes are ideal systems to test the synchronization hypothesis and to analyze strategies aimed at optimizing N use efficiency (NUE) in agroecosystems. In this context, the aim of the study was to analyze if the application of organic amendments of different C/N ratios, combined with mineral sources, can regulate N availability to the crop and reduce N losses from the agroecosystem. The hypothesis is that the organic substrates promote the immobilization of mineral N in the microbial biomass that becomes available later on by remineralization, controlling the amount of mineral N in the soil and therefore the synchronization with crop requirements.

Materials and methods

Study area

The study was conducted on a potato farm near the city of Mucuchies in the Andes of Mérida, Venezuela (8°46′N and 70°54′W, 2,960 m altitude). For the period 1972–2000, the mean annual rainfall was 640 mm, compared to an annual pan evaporation of 1,416 mm. The mean annual temperature was 11.1°C, with only minor variations throughout the year. The experiment was located on an alluvial fan with a mean slope of 20–30%. The soil is an *Anthropic Ustumbrept* according to the US Soil Taxonomy (Soil Survey Staff 1992), with a depth of approximately 1 m. Below the plow layer (30 cm) the soil is more compact and contains a higher proportion of stones.

Experimental design

To analyze the effect of different organic sources and their combinations on NUE and N losses, four fertilization treatments were applied, all of them receiving 25 g N m^{-2} of total N (considering the organic and mineral N forms). The description of the treatments, including the applied amounts of the different N sources, is presented in Table 1. MF, received only mineral fertilizer, P + F received a combination of poultry manure and mineral fertilizer, R + F received rice hulls and mineral fertilizer and in P + R + F poultry manure, rice hulls, and mineral fertilizer were combined. MF was considered as the control treatment, as the aim of the study was to evaluate if the addition of different organic sources can improve NUE and reduce losses compared to the sole addition of mineral N. The N applied in the mineral fertilizer was in the form of ammonium, 3.8 g N m^{-2} to all treatments as $(NH_4)_2HPO_4$ to homogenize P fertilization, and the

Table 1 Applied amounts (g DM m^{-2}) of the different amendments and their corresponding total N (g N m^{-2}) for the different fertilization treatments

Treatment[a]	Source applied (g DM m^{-2})				Amount of N applied (g N m^{-2})					%E[b]
	Poultry manure	Rice hulls	Ammonium		Poultry manure	Rice hulls	Ammonium		Total N	
			Phosphate	Sulfate			Phosphate	Sulfate		
MF	0	0	23.8	101.0	0	0	3.8	21.2	25.0	8.165
P + F	512	0	23.8	29.5	15.0	0	3.8	6.2	25.0	5.965
R + F	0	2,836	23.8	29.5	0	15.0	3.8	6.2	25.0	5.965
P + R + F	256	1,418	23.8	29.5	7.5	7.5	3.8	6.2	25.0	5.965

[a] Treatments: Mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hull and mineral fertilizer (R + F), and poultry manure, rice hull and mineral fertilizer (P + R + F). Mineral fertilizer sources were: Ammonium Phosphate = $(NH_4)_2HPO_4$ and Ammonium Sulfate = $(^{15}NH_4)_2SO_4$ with 10 atom % ^{15}N abundance

[b] %E corresponds to the atom % excess of the added mineral fertilizer (Ammonium Phosphate and Ammonium Sulfate). The mineral N in the poultry manure and rice hulls is not included for the calculation of this excess

rest as $(^{15}NH_4)_2SO_4$, with 10 atom percent ^{15}N abundance. The isotopic excess of the applied mineral fertilizer (%E of the mineral fertilizer) was 8.165% for the MF treatment and 5.965% for the rest of them (Table 1). To achieve a balanced NPK fertilization, 16.6 g K m^{-2} were applied to all treatments. Considering the mineral and extractable N measured in the soil, a conservative amount of N was applied, compared to the average used in the area (Corpoandes 1995).

Each treatment was applied to 17 square microplots (0.225 m^2), delimited with metallic sheets on all four sides, which were buried 0.3 m and surpassed the soil surface by 0.1 m, in order to prevent lateral N movements but allowing vertical water flux. A potato seed was planted at the center of each microplot. In total, seventeen soil–plant replicates per treatment were established. To homogenize surface soil (0–30 cm) and reduce variability between replicates, the total experimental area (25 m^2) was thoroughly plowed and all the soil was homogenized before installing the metallic sheets. Then the treatments were assigned to the microplots using a table of random numbers. The mineral and organic amendments, including the labeled ammonium sulfate, were homogeneously broadcast and incorporated into the 0–10 cm layer of each microplot at planting date.

Four samplings were carried out during crop development: (1) between crop emergence and the beginning of tuberization (33 days after planting, DAP); (2) at the end of shoot expansion (69 DAP); (3) at senescence (89 DAP), and (4) at tuber maturity (119 DAP). On each sampling date, all plant biomass and the entire soil of four microplots per treatment (0–30 cm layer) were collected, except for the last date when the remaining five microplots were used.

Agronomic practices

Planting was performed at a depth of 7 cm, using certified seeds of the Granola potato variety. A chemical pre-emergence weed control was conducted using atrazine. After emergence, manual weeding was carried out when necessary. Insects and nematodes were controlled with carbofuran at planting and afterwards symptomatic controls were applied. Five preventive and symptomatic chemical controls of *Phytophthora infestans* were carried out using curazin (active ingredient cymoxanil-mancozeb). Potato hilling was performed 65 DAP, heaping soil from inside each microplot (0–5 cm) around the potato plant. As the microplots were isolated from the surrounding soil by metallic sheets there was no risk of diluting the labeled fertilizer by mixing soil from outside the microplot during hilling. Four foliar applications of micronutrients (Mg, S, Cu, Zn, Mn at a dose by application of 20, 40, 5, 20, 5 g ha^{-1}, respectively) were carried out during crop development. Sixteen sprinkler irrigations (10 mm in average) were applied at a mean frequency of one per week, taking into consideration the amount of precipitation.

Initial soil characteristics

Three composite samples of the 0–30 cm soil layer, each consisting of three soil cores of 5.1 cm

Table 2 Main physical and chemical characteristics of the soil (0–30 cm)

Soil characteristic		Determination method
Sand (%)	49 (1)	Bouyoucos (1962)
Clay (%)	17 (1)	Bouyoucos (1962)
Silt (%)	34 (1)	Bouyoucos (1962)
Water retention—33 kPa (%)	22.7 (0.5)	Pressure plates (Klute 1986)
Water retention—1,500 kPa (%)	13.0 (0.8)	Pressure plates (Klute 1986)
Bulk density (g cm^{-3})	1.245 (0.04)	Excavation, gravimetry and volumetry
Stones (%)	10 (2)	Excavation, gravimetry and volumetry
pH (H_2O)	6.7 (0.1)	Potentiometry (IGAC 1978)
Organic C (g kg^{-1})	30.2 (1.3)	Walkley–Black (IGAC 1978)
Total N (g kg^{-1})	2.2 (0.1)	Kjeldahl (Bremner and Mulvaney 1982)
C/N	13.7 (0.7)	Calculation
Extractable N (mg kg^{-1})	150 (10)	K_2SO_4 extraction (Brookes et al. 1985)
NH_4^+-N (mg kg^{-1})	1.5 (0.3)	Distillation (Rojas and Castillo 1989)
NO_3^--N (mg kg^{-1})	30 (4)	Distillation (Rojas and Castillo 1989)
Microbial biomass N (mg kg^{-1})	26 (4)	Fumigation-extraction (Brookes et al. 1985)
P (mg kg^{-1})	510 (50)	Bray I (Rojas and Collazos 1989)
Exchangeable Ca (cmol kg^{-1})	14 (1.5)	Spectrophotometry (IGAC 1978)
Exchangeable Mg (cmol kg^{-1})	1.7 (0.1)	Spectrophotometry (IGAC 1978)
Exchangeable K (cmol kg^{-1})	2.1 (0.1)	Spectrophotometry (IGAC 1978)
Exchangeable Na (cmol kg^{-1})	0.04 (0.01)	Spectrophotometry (IGAC 1978)
CEC (cmol kg^{-1})	16.8 (1.0)	NH_4CH_3COO extraction (IGAC 1978)

Mean values and, in parentheses, the standard deviation of three soil cores samples

diameter, were collected before the installation of the microplots. The spatial coordinates from each core were selected using a table of random numbers. The samples were analyzed for the characteristics listed in Table 2. The soil had a sandy-loam texture, a pH close to neutrality and a high content of Ca due to the application of lime in previous agricultural cycles. Available P was also high due to frequent fertilization. Labile N forms (extractable N, NO_3^--N and N in the microbial biomass) represented 8% of the total N. The initial amount of mineral N in the soil was 31.5 mg kg^{-1}, equivalent to 105 kg ha^{-1}, which is not enough to fulfill crop requirements (150–300 kg N ha^{-1} according to Van Delden et al. 2003).

Organic amendments

Dry industrial poultry manure, commercialized in packets, was used for the experiment. Rice hulls with low water content were bought directly at rice factories. To characterize these organic amendments and calculate the required doses for the different treatments, they were analyzed for total N, total extractable N, mineral N and total organic C. The results of these analyzes confirm their very contrasting quality (Table 3).

Soil and plant nitrogen

For establishing the N budget, a depth of 0–30 cm was selected, based on a preliminary study of crop root distribution in the profile. This study was carried out in an adjacent plot cultivated with the same potato variety, where four soil columns were taken at crop maturity using a frame of 25 × 25 cm and divided in layers of 0–15, 15–30 and 30–45 cm. In average 95 ± 3% of the roots were above 30 cm depth (82% between 0–15 and 13% between 15–30 cm).

On each sampling date all the soil from the 0–30 cm layer of each selected microplot ($\approx$ 100 kg) was sieved to 4 mm in the field and mixed thoroughly before collecting a homogeneous sub-sample of approximately 1 kg in which total N, NH_4^+-N, NO_3^--N, total extractable N, microbial N and the $^{15}N/^{14}N$ isotope

Table 3 Mean values, and in parentheses the standard deviation ($n = 3$), of C and N content (dry matter basis) of poultry manure and rice hulls

	Poultry manure	Rice hulls	Determination method
Extractable N (g kg^{-1})	11.0 (0.1)	0.22 (0.02)	Extraction (Brookes et al. 1985)
NH_4^+-N (mg kg^{-1})	5,190 (140)	71 (6)	Distillation (Rojas and Castillo 1989)
$[NO_3^- + NO_2^-]$-N (mg kg^{-1})	757 (32)	20 (2)	Distillation (Rojas and Castillo 1989)
Total N (g kg^{-1})	29.3 (0.5)	5.3 (0.2)	Kjeldahl (Bremner and Mulvaney 1982)
Organic C (g kg^{-1})	307 (4)	486 (4)	Walkley–Black (IGAC 1978)
C/N	10.5 (0.3)	91.7 (4.0)	Calculation

ratio of each fraction were determined. At the same time, the entire plant was collected and dry biomass of the different organs was determined. Then a composite sample per plant (including all organs) was prepared for the determination of total and ^{15}N. Soil and plant samples were ground and carefully homogenized before being analyzed.

Nitrogen inputs from rainfall and irrigation

Every week rainfall samples were collected for N analysis in three pluviometers of 15 cm diameter, located in the experimental area. The N content of irrigation water was measured in samples collected from the irrigation tanks and the water amount was measured using a set of 15 cm diameter collectors scattered throughout the experimental area.

Drainage and leached nitrogen

To determine the amount and N content of the water drained at 30 cm soil depth, six zero-tension closed lysimeters by treatment were installed next to the microplots. Each lysimeter consisted of a metal cylinder of 0.225 m^2 surface area (the same as for the microplots) and 35 cm depth, open to the atmosphere at the top but closed at the bottom, where percolating water could flow through a hose to a 20 L water container located 5–6 m downhill. The lysimeters were installed on level ground and a layer of 2 cm of washed coarse sand was placed at the bottom, followed by 30 cm of plowed soil from the site, which was packed with the same bulk density and stone proportion of the surrounding soil. The upper border of the lysimeters was 5 cm above soil surface to exclude runoff water for entering or exiting. The decision to exclude runoff both in the lysimeters as in the microplots was made because runoff accounts for only 1% of the water input by rainfall and irrigation on these slopes cropped with potatoes (Díaz 2009). A potato seed was planted in the center of each lysimeter, and the corresponding fertilization treatment was applied using the same procedure as for the microplots.

At weekly intervals, percolated water was measured and a 500 mL aliquot from each lysimeter was collected and kept frozen for N analysis. Total leached N (soluble and mineral) and its ^{15}N/^{14}N ratio were analyzed in four composite water samples prepared from the weekly samples to match the intervals between microplots sampling dates.

Zero-tension lysimeters filled with disturbed soil can in some cases produced biases in the estimation of drainage due to changes in soil structure that can cause preferential flow pathways and additionally a saturated zone needs to be created before water can drain out. However, the high sand content of this soil (49%) minimizes the effect of the discontinuity between the soil column and the sand layer at the bottom of the lysimeter. On the other hand, an advantage of zero-tension lysimeters is that they collect only the gravitational water while tension lysimeters collect different portions of soil water depending on the tension. Another advantage is that water composition is not modified as occurs in tension lysimeters where the ceramic porous materials can affect the chemical composition of the solution due to processes of ionic exchange. All methods to measure drainage are subject to different kinds of errors (Arauzo et al. 2010) and in our case given the high stone content of the soil, the use of zero-tension lysimeters filled with repacked soil was the best option, even if some error in the estimation of the absolute values occur, they can be used to compare the leached N between treatments.

To evaluate the performance of the lysimeters, the measured values were compared to drainage estimations based on the establishment of a daily water budget, in mm, for the 0–30 cm soil layer. The following equation was used:

$$P + I = \Delta H + ETc + D$$

where P is precipitation, I is irrigation, ΔH is the daily variation of soil water storage (recalculated day by day from the initial value), D is drainage calculated as the soil water exceeding field capacity and ETc is crop evapotranspiration, defined as the sum of evaporation from the soil, evaporation from crop interception and crop transpiration. ETc was evaluated using the FAO-56 procedure (Allen et al. 1998) by means of the equation:

$$ETc = Kc\ Ks\ ETo$$

where Kc is a crop coefficient calculated by interval (Kc_{ini}:1, Kc_{mid}:1.15 and Kc_{end}:0.75, according to Allen et al. 1998). The duration of the intervals was determined as a function of crop LAI for each treatment. Ks is a water stress coefficient that acts as a reduction factor dependent on available soil water. This factor varies from 1 at field capacity to 0 at wilting point, assuming a linear reduction that in potatoes begins when soil water decreases below 0.35 of the total available soil water (Allen et al. 1998). ETo is the reference evapotranspiration estimated according to the FAO-56 Penman–Monteith equation. In the estimation of ETo, Kc and Ks, meteorological daily data (temperature, precipitation, relative humidity, wind speed, solar radiation), latitude, altitude, day of the year and soil data (wilting point, field capacity, bulk density, stoniness) were used (Allen et al. 1998). Meteorological data were collected from a weather station (Campbell Instruments) installed in the experimental area.

Nitrification

Soil nitrification was estimated using the equation of Kandeler (1996):

$$\%Nit = 100 \times (NO_3^-\text{-}N_{t_i} - NO_3^-\text{-}N_{t_{i-1}}) / [NH_4^+\text{-}N_{t_{i-1}} + (Nmin_{t_i} - Nmin_{t_{i-1}})]$$

where: %Nit is the relative nitrification during the considered time interval. $NO_3^-\text{-}N_{t_i}$ and $NO_3^-\text{-}N_{t_{i-1}}$ are the amount of nitrate and nitrite at t_i and t_{i-1}; $Nmin_{t_i}$ and $Nmin_{t_{i-1}}$ are the amount of mineral N (NO_3^--N + NH_4^+-N) at t_i and t_{i-1}, respectively and $NH_4^+\text{-}N_{t_{i-1}}$ is the amount of ammonium at t_{i-1}. The time intervals corresponded to the sampling dates of the microplots.

Chemical and physical analysis

Total N and ^{15}N were determined in air dried soil samples ground to pass a sieve of 125 µm. The measurements were carried out by combustion, gas chromatography and isotopic ratio mass spectrometry (Scrimgeour and Robinson 2003). Plant total N was also measured in ground samples using the Kjeldahl method (Bremner 1965) and its atom %^{15}N abundance by optical emission spectrometry (Axmann et al. 1990). Soil microbial biomass N was measured using the fumigation-extraction method (Brookes et al. 1985). A fumigated and a control soil were extracted with 0.5 M K_2SO_4 and total N was measured in the supernatants by Kjeldahl digestion and distillation (Bremner and Mulvaney 1982). The N in the microbial biomass was calculated as the difference between fumigated and control extracts divided by a K_{EN} factor of 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996). The total N content of the 0.5 M K_2SO_4 extract in the non-fumigated soil is referred to in the text as "total extractable N". The NH_4^+-N was measured in fresh soil 0.5 M K_2SO_4 extracts by distillation of the supernatant after addition of MgO. For the determination of NO_3^--N a second distillation was carried out after the addition of Devarda's alloy to reduce NO_3^- to NH_4^+ (Rojas and Castillo 1989).

Nitrogen in rainfall, irrigation and percolation water was measured on aliquots, concentrated by evaporation after addition of H_2SO_4 to prevent losses and measured by Kjeldahl digestion and distillation (Bremner and Mulvaney 1982).

All the distillates from the microbial biomass, mineral N and percolation water were collected in H_2SO_4 solution and N was determined by back titration with NaOH. After titration, the solution was adjusted to pH 3–4 with concentrated H_2SO_4 to prevent N losses, and the samples were evaporated to obtain $(NH_4)_2SO_4$ crystals whose Atom %^{15}N abundance was determined by optical emission spectrometry (Guiraud and Fardeaux 1980; Preston 1999).

Labeled nitrogen calculations

The ^{15}N isotopic excess (%E) was calculated from atom %^{15}N abundance (%A) as $\%E = \%A - \%a$ (Guiraud 1984), where %a is the natural isotope abundance measured in a reference standard with the same methodology used for %A.

At each sampling date, the percentage of N derived from the mineral fertilizer (%Ndff) in the plant biomass, in the different soil compartments and in drained water, and the absolute amount of N from the mineral fertilizer (Nf) in each compartment were calculated according to Halitligil et al. (2002) as:

$$\begin{aligned}&\%\text{Ndff compartment}\\&\quad = (\%\text{E compartment}/\%\text{E fertilizer}) \times 100\\&\text{Nf compartment}\left(\text{g N m}^{-2}\right)\\&\quad = \%\text{Ndff} \times \text{N compartment }\left(\text{g N m}^{-2}\right)/100\end{aligned}$$

and therefore the percentage of the N applied in the mineral fertilizer (%Nf), found in each compartment was calculated as:

$$\begin{aligned}&\%\text{Nf compartment}\\&\quad = \big(\text{Nf compartment}/\text{N applied in the mineral}\\&\quad\ \text{fertilizer}\big) \times 100\end{aligned}$$

The amount of soil extractable organic N derived from the fertilizer in all treatments was calculated as the difference between the total amount of extractable Nf and the amount of mineral Nf (Zhong and Makeschin 2003). The amount of non-extractable organic N from the fertilizer was estimated as the total Nf in the soil minus all the other Nf soil fractions (mineral, microbial and extractable organic N). Unaccounted Nf was calculated as the amount of N applied in the mineral fertilizer minus the amount of N from the fertilizer (Nf) found in the plant–soil system and in the drained water.

Nitrogen budget

A N budget that includes the change in compartments and the fluxes of labeled and non-labeled N between planting and harvest was calculated for each treatment for the 0–30 cm soil layer. Five compartments were included: plant N (N_{plant}), soil mineral N (N_{min}), soil microbial biomass N (N_{mb}), soil extractable organic N (N_{eo}) and soil non-extractable organic N (N_{neo}). The following fluxes were calculated: input of mineral N from the fertilizer ($N_{fertilizer}$), the organic amendments ($N_{min\ org\ amendment}$), and in rainfall and irrigation (N_{water}), input of organic N from the organic amendments, either extractable ($N_{eo\ org\ amendment}$) and non-extractable ($N_{neo\ org\ amendment}$), plant uptake (N_{uptake}), net mineralization-immobilization ($N_{mineralized}$), net decomposition-synthesis of non-extractable ($N_{neo\ decomposed}$) and extractable organic N ($N_{eo\ decomposed}$), losses by leaching ($N_{leached}$) and losses in gaseous form ($N_{gaseous\ losses}$). For a better understanding of the conceptual model see the N balances presented in Fig. 4.

The change in the size (Δ) of each compartment can be expressed as:

$$\begin{aligned}&\Delta\text{N-plant} = N_{uptake}\\&\Delta\text{N-min} = N_{fertilizer} + N_{min\ org\ amendments} + N_{water}\\&\quad + N_{mineralized} - N_{uptake} - N_{gaseous\ losses} - N_{leached}\\&\Delta\text{N-mb} = N_{neo\ decomposed} + N_{eo\ decomposed} - N_{mineralized}\\&\Delta\text{N-eo} = N_{eo\ organendment} - N_{eo\ decomposed}\\&\Delta\text{N-neo} = N_{neo\ organendment} - N_{neo\ decomposed}\end{aligned}$$

The delta of all the compartments was determined directly from measured values, except for ΔN-neo, which was estimated by difference in the budget. All inputs from the mineral fertilizer and from the organic amendments were measured. $N_{leached}$ was measured using the lysimeters. $N_{gaseous\ losses}$ of the mineral fertilizer were considered equal to the unaccounted Nf. Gaseous losses of the non-labeled N were not considered in the balance, so $N_{gaseous\ losses}$ corresponded to those from the mineral fertilizer. The rest of the fluxes were calculated using the equations for the deltas: N_{uptake} from ΔN-plant, $N_{mineralized}$ from ΔN-min, $N_{eo\ decomposed}$ from ΔN-eo and $N_{neo\ decomposed}$ from ΔN-mb.

Indices of N use efficiency

The crop N use efficiency was evaluated calculating the apparent recovery of the N applied in mineral and organic sources (apparent NUE, Mosier et al. 2004):

$$\text{Apparent NUE } (\%) = \left(\left(N_{plant} - N_{soil}\right)/N_{applied}\right) \times 100$$

where $N_{applied}$ is the total amount of applied N (25 g m^2), N_{plant} is the N measured in plant biomass at harvest, and N_{soil} is the amount of N_{plant} coming from the initial soil N and calculated in the control treatment (MF) as the total N plant uptake minus the N plant uptake from the labeled mineral fertilizer. The initial soil N is the amount of N present in the soil before the application of treatments. This procedure to calculate NUE considers the "priming effect" or "real added nitrogen interaction" (Hart et al. 1986) that is ignored when NUE is calculated by the traditional non-isotopic difference method that uses an unfertilized treatment as control.

Also the nitrogen use efficiency of the mineral fertilizer (NUEf) was calculated, as the percentage of the added N from the fertilizer found in plant biomass at harvest.

To evaluate the effect of the treatments not only on crop N uptake but also on N losses, two additional indices were proposed. The first one is the N use to total losses index (NUTLI), which was calculated as the ratio between the total amount of N in crop biomass at harvest and the total amount of N lost from the agroecosystem (leaching plus unaccounted N). The second one is the nitrogen use to leached index (NULEI), calculated as the ratio between the total N in plant biomass at harvest and the total amount of leached N. As for the total applied N, NUTLIf and NULEIf were calculated considering only the uptake and losses of the labeled mineral fertilizer.

Statistical analysis

The statistical analysis was carried out using the package STATISTICA 4.0. Treatments were compared by one-way ANOVA after testing for normality and homogeneity of variances. Tukey's honestly significant difference test (HSD) at $P < 0.05$ was used for post-hoc comparisons of the means.

Results

Plant biomass and nitrogen

At harvest, tuber yield, total plant biomass, and total plant N were significantly different among treatments, following the sequence, R + F > P + R + F > P + F and MF (Table 4). Despite the same total amount of N applied to all treatments, tuber yield, plant biomass, and N accumulation in the R + F treatment, were 36.7, 36.1 and 24% higher, respectively, when comparing to the control (MF).

Root biomass and the ratio of root to shoot biomass were significantly higher in the treatments with rice hulls, but only on the first sampling date (Table 4). For the other three samplings the differences between treatments were not significant (data not shown).

Processes of the N cycling

Nitrification

During the first month of crop development, the percentage of nitrification of the total and labeled NH_4^+ was very high for all treatments (99.5–100%, data not shown). Right after fertilization the amount of NO_3^--N + NO_2^--N represented 30, 47, 51 and

Table 4 Mean values, and in parentheses the standard deviation, of: tuber yield, total plant biomass and total N in plant biomass at harvest ($n = 5$), shoot/root ratio and root biomass at the first sampling date, 33 DAP ($n = 4$), for the different treatments

Parameters	Treatments[a]			
	MF	P + F	R + F	P + R + F
Tuber yield (kg DM m^{-2})	1.20 (0.07) c	1.23 (0.06) c	1.64 (0.05) a	1.51 (0.06) b
Total plant biomass (kg DM m^{-2})	1.44 (0.09) c	1.45 (0.05) c	1.96 (0.08) a	1.78 (0.09) b
N plant uptake (g N m^{-2})	22.8 (0.9) c	23.8 (0.6) c	28.2 (0.8) a	26.6 (0.7) b
Root/shoot ratio at 33 DAP	0.19 (0.02) c	0.16 (0.02) c	0.30 (0.02) a	0.24 (0.02) b
Root biomass at 33 DAP (g m^{-2})	1.1 (0.2) c	0.8 (0.2) c	2.2 (0.2) b	3.2 (0.3) a

Different letters indicate significant differences among treatments (Tukey HSD, $P < 0.05$)

[a] Treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hull and mineral fertilizer (R + F), and poultry manure, rice hull and mineral fertilizer (P + R + F)

49% of the total mineral N for the treatments MF, P + F, R + F and P + R + F, respectively. However, at 33 DAP this proportion increased to 98% in all treatments, and remained higher than 95% afterwards, providing evidence of high nitrification rates.

Plant uptake

Most of the plant N uptake took place during the second and third month of potato growth (Fig. 1a). Afterward, the quantity of N in plant biomass remained almost constant. Nitrogen accumulation was highest in the treatments with rice hulls.

Mineralization and immobilization

Soil mineral N decreased considerably in all treatments during the first month after potato planting, and remained very low afterwards, especially in the R + F treatment, where it was significantly lower than in the other treatments (Fig. 1b). Simultaneously to the decrease of mineral N, extractable organic N

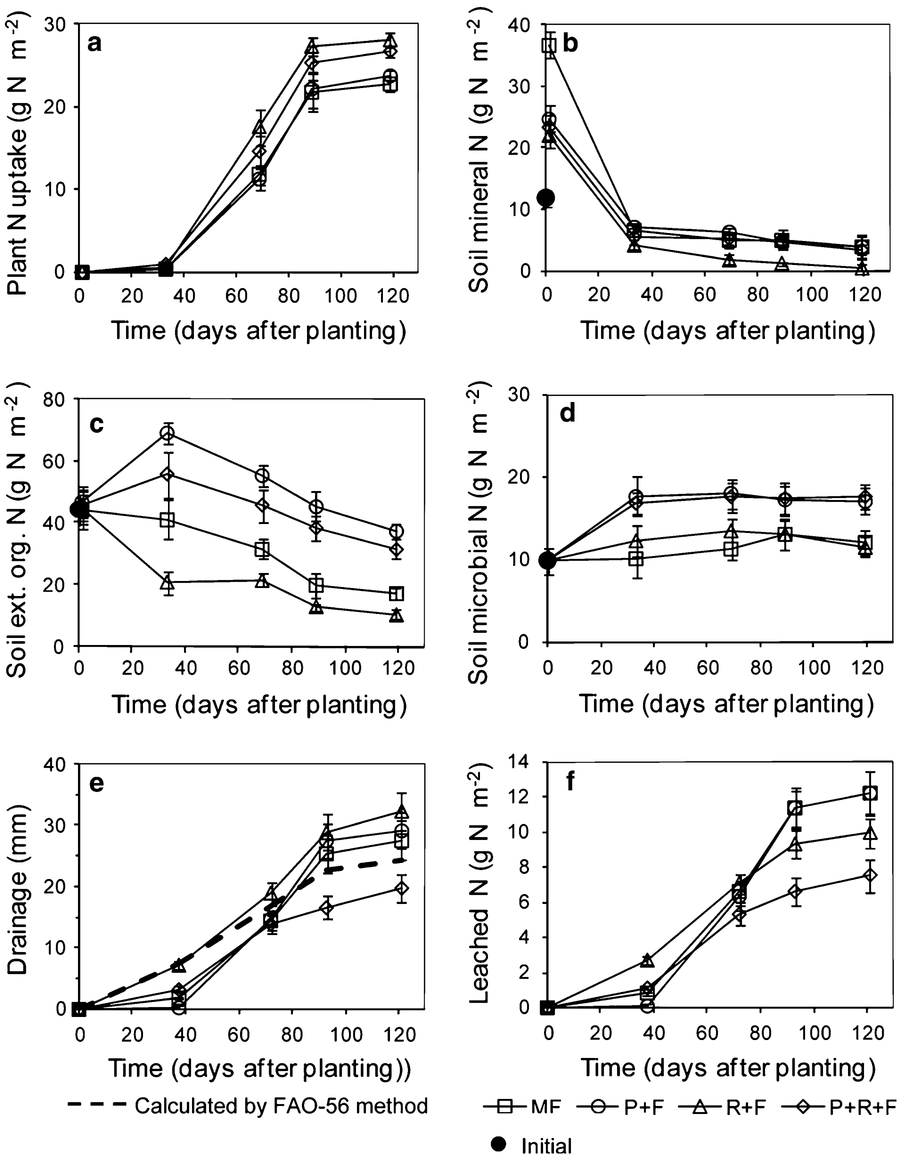

Fig. 1 Course of: plant N (**a**); soil mineral N (**b**); soil extractable organic N (**c**); soil microbial N (**d**); cumulative drainage measured in the lysimeters and mean cumulative drainage for all treatments calculated by the water balance method (**e**) and cumulative losses of N by leaching (**f**), for the different treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hulls and mineral fertilizer (R + F), and poultry manure, rice hulls and mineral fertilizer (P + R + F). *Points* represent mean values and *bars* represent the standard deviation ($n = 4$)

increased in the treatments with poultry manure but not in the others, then the trend is a decrease in all treatments (Fig. 1c). Net N immobilization in the microbial biomass mainly occurred during the first month of crop development and was again highest in the treatments with poultry manure (P + F and P + R + F), lower in the R + F treatment and null in the MF treatment (Fig. 1d).

Water drainage and N leaching

Total precipitation during the whole period of cultivation (117 days) was 249 mm, and total irrigation 160 mm. For the same period, cumulative drainage at 30 cm depth, obtained from the lysimeters, was only 27.6, 29.3, 32.3 and 19.8 mm for MF, P + F, R + F and P + R + F, respectively (Fig. 1e), with an average of 27.5 mm. The estimated drainage using the FAO-56 method (Allen et al. 1998) was 24.5, 25.0, 24.0 and 23.7 mm respectively, with an average of 24.3 mm. Both methods produced very close estimates of the cumulative drainage (3 mm difference in the mean values) and similar temporal dynamics (Fig. 1e), giving a positive validation of the performance of the lysimeters. Consequently, for the purposes of this study the obtained drainage values provide a valid estimation of N leaching losses.

Despite low drainage, large N losses by leaching were detected, which accounted for 7–12 g N m^{-2}, depending on the treatment (Fig. 1f). Averaged throughout the whole period and all the treatments, the mean concentration of N in percolation water was 400 mg L^{-1} (range 105–554 mg L^{-1}). In comparison, the mean amount of soil mineral and extractable N for all the treatments was 44 g N m^{-2} (range 17–75 g N m^{-2}, Fig. 1b, c). At a bulk density of 1.245 g cm^{-3} and a soil water saturation of 25% v/v, this value corresponds to an approximate soil solution concentration of 470 mg L^{-1} (range 182–803 mg L^{-1}). Therefore, the concentration of N in the drainage water (400 mg L^{-1}) was lower than that calculated in the soil solution (470 mg L^{-1}) as to be expected considering that part of the mineral and extractable nitrogen is protected in the exchangeable complex.

Fate of the N added by mineral fertilization

The dynamics of the N added in the mineral fertilizer, expressed as changes in the percentage of the total applied fertilizer (%Nf) during the cropping period showed that (Fig. 2): (1) the percentage of unaccounted labeled N (at least partly attributable to gaseous losses) was significantly higher in the treatments MF and R + F than in those with poultry manure (P + F and P + R + F) and, in all treatments, most of it ($\approx$80%) disappeared during the first month; (2) the percentages of labeled N in plant biomass and lost by leaching increased gradually during crop development as labeled N in the soil compartments decreased; (3) the percentage of total labeled N in mineral forms decreased from 100% at planting to less than 6% after 33 days; meanwhile, labeled N in organic forms increased; (4) at day 33, the extractable soil organic N was the predominant form, and afterwards this form decreased while non-extractable organic N increased; and (5) the percentage of labeled N in the microbial biomass increased during the first interval and then was variable, with a tendency to be lower in the MF treatment.

The distribution of labeled N at harvest (Fig. 3) revealed that: (1) the percentage of labeled N found in the plant biomass was lower ($P < 0.05$) in the MF treatment than in R + F and P + R + F, and it was not significantly different in P + F, compared to the other treatments; (2) the unaccounted N decreased ($P < 0.05$) in the order MF (52%) >R + F (39%) >P + R + F (14%) and P + F (12%); (3) the leaching losses were lowest for P + R + F (3%) and highest for R + F (10%), (4) the labeled N that remained in the soil at the end of the experiment was significantly higher in the treatments P + R + F and P + F (61%) than in R + F (26%) and MF (25%). Of this residual labeled soil N, mineral N represented only a very small fraction in all treatments.

Nitrogen budget

The N budget for the different treatments, considering the fluxes of total N (labeled and non-labeled) between planting and harvest is presented in Fig. 4. The inputs of N by precipitation (0.3 g N m^{-2}) and irrigation (0.1 g N m^{-2}) were very low and therefore were not considered in the N balance. The input of N by fertilization (25 g m^{-2}) entered in different proportions into the mineral, the extractable organic and the non-extractable organic compartments, depending on the treatment (Fig. 4). Leaching was the highest output flux, except for the MF treatment, where

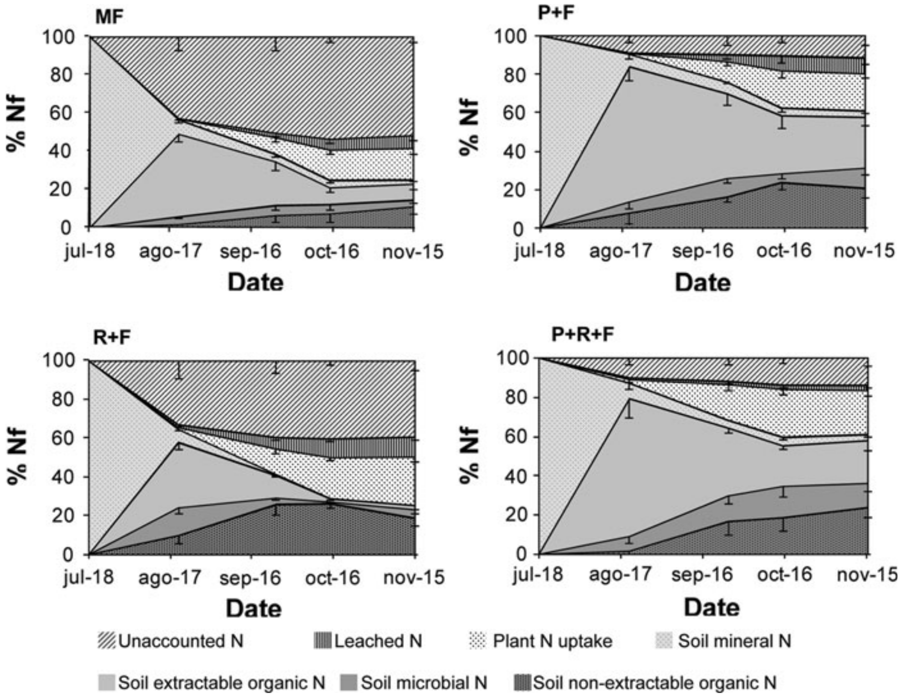

Fig. 2 Percentage of the applied labeled mineral N (%Nf) found between planting and harvest in the total plant biomass; in the following soil compartments: mineral N, extractable organic N, microbial biomass N, and non-extractable organic N; lost by leaching and unaccounted N, for the different treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hulls and mineral fertilizer (R + F), and poultry manure, rice hulls and mineral fertilizer (P + R + F). *Bars* represent the standard deviation ($n = 4$)

unaccounted N and leaching losses were similar. The treatments with rice hulls had the lowest leaching losses, whereas those with poultry manure had the lowest unaccounted N. Assuming that most of the unaccounted N was lost to the atmosphere and that the rates of loss of non-labeled N are similar to those of labeled N, an estimation of the gaseous losses of non-labeled N gives figures, in g N m^{-2}, of 1.1 (MF), 0.7 (P + F), 0.8 (R + F) and 0.6 (P + R + F). As these values are so low, they were not considered in the N budget calculation.

The processes dominating the N cycling were: plant uptake, net mineralization and decomposition of extractable organic N. Plant uptake was significantly higher in the treatments with rice hulls (P + R + F and specially R + F), compared to the other treatments. Net mineralization predominated over net immobilization when the whole cultivation period was considered, but there were two differentiated phases: (1) during the first month of crop development, 50% (MF), 66% (P + F), 51% (R + F) and 64% (P + R + F) of the total mineral N and 49% (MF), 84% (P + F), 58% (R + F) and 79% (P + R + F) of labeled N was transformed into organic forms (microbial biomass, extractable and non- extractable organic N); and (2) between 33 days and harvest, 26–56% of the N previously immobilized was remineralized.

Concerning the organic compartments, the extractable fraction decreased in the four treatments, being the main source of N for the crop, while the non-extractable organic compartment increased in R + F and to a lesser extent in MF and experienced little change in the treatments with poultry manure.

Nitrogen use efficiency

Use efficiency of the total applied N

The apparent NUE of the applied N was significantly different among treatments (Table 5). When all N was added in mineral form or combining mineral N with poultry manure, the recovery by the crop was less than 20%, but increased to 38% in the treatment with rice hulls (P + F) and to 32% in P + R + F. Another aspect to be considered in the assessment of N use efficiency is the amount of N lost from the agroecosystems (Table 5). Total losses of N were very high for the MF treatment (25 g N m^{-2}), followed by R + F (13.8 g N m^{-2}), P + F (13.4 g N m^{-2}) and P + R + F (8.8 g N m^{-2}). While total losses were similar for both R + F and P + F, the pathways were not, as

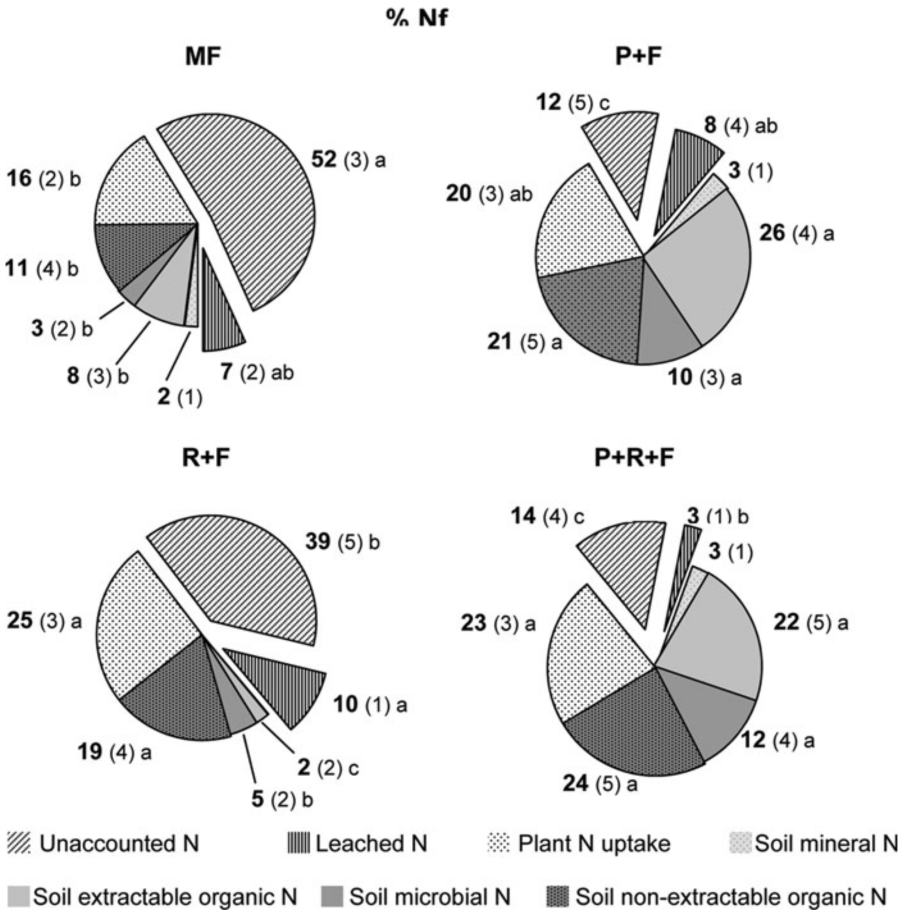

Fig. 3 Percentage of the applied labeled mineral N (%Nf) found at harvest in the total plant biomass; in the following soil compartments: mineral N, extractable organic N, microbial biomass N and, non-extractable organic N; lost by leaching and unaccounted N, for the different treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hulls and mineral fertilizer (R + F), and poultry manure, rice hulls and mineral fertilizer (P + R + F). *Numbers* represent the average percentage and, in parentheses, the standard deviation ($n = 5$). *Different letters* indicate significant differences (Tukey's HSD, $P < 0.05$) between treatments

R + F had higher gaseous losses while P + F had higher leaching losses (Fig. 4).

The proposed indices NUTLI and NULEI, which consider simultaneously the uptake by the crop and the losses, were significantly different among treatments: P + R + F > R + F, P + F > MF for NUTLI and P + R + F > R + F > P + F and MF for NULEI, showing that the combination of substrates of different qualities represented the best alternative.

Use efficiency of the mineral fertilizer

NUEf was significantly lower in MF (16%) than in R + F (25%) and P + R + F (23%) (Table 5). For P + F, NUEf (20%) did not significantly differ from that of the other treatments. Also the percentage of plant N derived from mineral fertilizer (%Ndff) was higher in MF (18%) than in the other treatments (between 8 and 9%). NUTLIf was highest for the treatments with poultry manure (P + R + F and P + F), followed by R + F and MF, while NULEIf was highest for the treatment combining poultry manure and rice hulls (P + R + F), compared to the others.

Discussion

The synchronization hypothesis proposes that a better match between crop N requirements over time and N availability in the soil, and consequently a higher N plant uptake and a reduction of N losses, can be obtained by combining organic substrates of different quality (Swift 1984, 1987; Myers et al. 1994). In this experiment the lowest losses occurred in the treatment which combined poultry manure and rice hulls (P + R + F), but it was the sole addition of the lower quality substrate (rice hulls, R + F) that promoted a higher N crop uptake and consequently a higher tuber

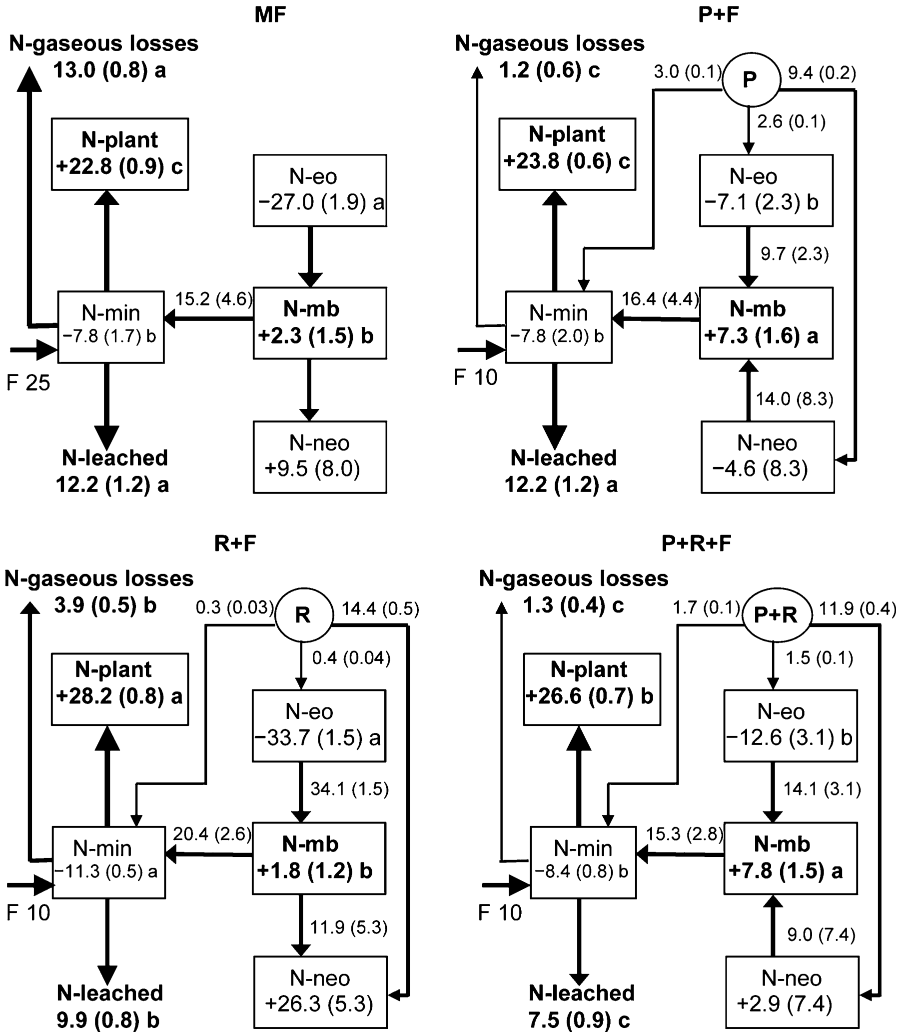

Fig. 4 Nitrogen balance between planting and harvest considering all N (labeled and non-labeled) for the different treatments. Arrows represent the net N fluxes in g N m^{-2} and boxes represent the net variation in the compartments (N-plant = plant N, N-min = mineral N, N-mb = microbial biomass N, N-eo = extractable organic N, N-neo = non-extractable organic N). N gaseous losses were considered equal to unaccounted Nf. In the balance, the amount of different forms of N (mineral, extractable organic and extractable non-organic) added in each source (F = mineral fertilizer, P = poultry manure, R = rice hulls) were taken into account. Numbers are mean values and in parentheses the standard deviation. Different letters indicate significant differences (Tukey's HSD, *P* = 0.05) between treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hulls and mineral fertilizer (R + F), and poultry manure, rice hulls and mineral fertilizer (P + R + F)

yield. The differences among the treatments do not seem to be due to the deficit of other nutrients as the treatments with rice hull produced the larger effect on potato production but this substrate contains minor amounts of other nutrients compared to the poultry manure (Machado 2005). Furthermore, to reduce possible nutrient limitations, P, K and micronutrients were added to all the treatments.

A possible explanation for the higher yields obtained in the rice hull treatments is that root development was stimulated by the addition of this substrate, allowing a better soil exploration and N uptake. This stimulation can be explained by: (1) a decrease in soil bulk density (calculated as 19% for the first 10 cm of the soil profile), which could reduce physical resistance to root penetration, and (2) a

Table 5 Mean values, and in parentheses the standard deviation ($n = 5$), of indicators of the agroecosystem performance at harvest: apparent use efficiency of the total N applied (apparent NUE), fertilizer N use efficiency (NUEf), % of plant nitrogen derived from the fertilizer (Ndff), nitrogen use-total losses index for the fertilizer (NUTLIf); nitrogen use-leaching losses index for the fertilizer (NULEIf); total N losses; nitrogen use total losses index (NUTLI) and nitrogen use leaching losses index (NULEI), for the different treatments

Parameters	Treatments[a]			
	MF	P + F	R + F	P + R + F
Apparent NUE (%)	16 (2) b	20 (2) b	38 (3) a	32 (3) a
NUEf (%)	16 (2) b	20 (3) ab	25 (3) a	23 (3) a
Ndff (%)	18 (2) a	8 (1) b	9 (2) b	9 (1) b
NULEIf	2.4 (0.9) b	2.5 (0.9) b	2.5 (0.5) b	9.0 (1.0) a
NUTLIf	0.3 (0.1) c	1.0 (0.3) a	0.5 (0.1) b	1.4 (0.4) a
Total N losses (g N m^{-2})	25.2 (2.0) a	13.3 (1.8) b	13.8 (1.3) b	8.8 (1.3) c
NULEI	1.9 (0.2) c	2.0 (0.2) c	2.8 (0.3) b	3.6 (0.4) a
NUTLI	0.9 (0.1) c	1.8 (0.3) b	2.0 (0.2) b	3.0 (0.4) a

Different letters indicate significant differences between treatments (Tukey HSD, $P < 0.05$)

[a] Treatments: mineral fertilizer (MF); poultry manure and mineral fertilizer (P + F); rice hull and mineral fertilizer (R + F), and poultry manure, rice hull and mineral fertilizer (P + R + F)

moderate initial N deficit, due to the lower amount of available N in rice hull, which could induce a change in the biomass allocation pattern of the crop.

The positive effect of the addition of rice hulls on plant N accumulation allowed a higher efficiency in the use of both mineral fertilizer (NUEf) and total added N (apparent NUE). Nevertheless, for all treatments, the NUEf was very low (16–25%). Also the proportion of plant N coming from the fertilizer was very low (%Ndff: 8–9% for the treatments P + F, R + F and P + R + F and 18% for MF). These results indicate that, in all treatments, plants used more N from other sources than from the applied mineral fertilizer. In the MF treatment, in which all the N (25 g N m^{-2}) was applied in mineral form, the value of 18% Ndff at harvest is low compared to the results of other studies. For example, at a similar fertilization rate, %Ndff values of 70% were reported in another area of the Venezuelan Andes (Abreu et al. 2007); 53–60% using a fertilization of 140 kg N ha^{-1} in Canada (Tran and Giroux 1991) and 60–67% with a fertilization of 400–1,000 kg N ha^{-1} in Turkey (Halitligil et al. 2002). In the MF treatment, most of the 22.8 g N m^{-2} absorbed by the crop came from the pool of 11.8 g N m^{-2} mineral nitrogen and of 44.3 g N m^{-2} extractable organic N present in the soil before the application of the treatments. Also most of the added N in this treatment was lost from the soil before the period of rapid N uptake, which explains the N deficit of the crop (24% less uptake than in R + F). For the other treatments the apparent NUE indicates that 66–79% of the N taken up by the crop came from the initial soil N, which was essential for obtaining the high yields measured (70–90 Mg ha^{-1} of fresh tuber yield), despite the important losses of added N.

Possible causes for the low recovery of the added N by the crop are the high N losses as well as the immobilization and the high availability of N from other sources. Most of the unaccounted N was considered as lost in gaseous forms because: (1) no dilution of the labeled N was possible as the microplots were isolated from the surrounding soil; (2) the important and consistent differences between treatments indicate that methodological errors are not the main cause of this unaccounted N; and (3) the sandy-loamy soil texture, its pH close to neutrality and the high soil temperatures during the day are favorable conditions for volatilization and denitrification. The unaccounted N was significantly higher in MF (52% of the added Nf) and R + F (39%), compared to the poultry manure treatments P + F (12%) and P + R + F (14%). The losses in MF and R + F are higher than the values reported by several authors for potato: 32% of the added N lost to the atmosphere (Abreu et al. 2007), and total N losses of 12–40% reported by Maidl et al. (2002); 19% reported by MacDonald et al. (1997) and 14–18%

obtained by Halitligil et al. (2002). Ruser et al. (1998) indicated losses of 11–16% of the fertilizer N as N_2O in potato, significantly higher than in other crops.

In the poultry manure treatments, where lower unaccounted N was found during the first period of crop development, more of the added labeled N was found in the microbial biomass and in extractable organic forms, revealing a higher immobilization. This immobilization is probably related to the high content of organic labile compounds in poultry manure (Beloso 1991; Machado 2005), which may have stimulated the activity of the soil microbiota and the transformation of part of the mineral N into organic products or microbial metabolites. The role of microorganisms producing extractable N is supported by the results of Appel and Mengel (1993), who found that extractable organic N reflected the microbial activity in the soil, and Friedel et al. (2001), who stressed the role of the microbial biomass as a source-sink for extractable organic N.

The amounts of residual labeled N found in the soil of the poultry manure treatments at harvest, 60.7% in P + F and 60.8% in P + R + F, are very high compared to 25 and 36% in the MF and R + F treatments respectively, and compared to the 39% obtained by Abreu et al. (2007), 46% by Halitligil et al. (2002), 21% by MacDonald et al. (1997) and 19.5–24.6% reported by Maidl et al. (2002) for potato crops. Considering that a significant fraction of this residual Nf was found in the extractable organic compartment and in the microbial biomass, it is reasonable to suppose that the high content of extractable N measured at the beginning of the experiment was a consequence of the poultry manure added in previous cultivation cycles.

In general, relatively low leaching losses of labeled N were found, only 3–10% of the added mineral N. These figures are similar to the 6% found by Abreu et al. (2007) but much lower than the 20–28% reported by Ünlü et al. (1999) in Turkey with a fertilization of 400–1,000 kg N ha^{-1}, respectively. Nevertheless, total losses through leaching, considering unlabeled N, were high (7.5–12.2 g N m^{-2}), with lower values for the rice hulls treatments (P + R + F and R + F), compared to P + F and MF, because this substrate promoted N immobilization (due to its high C/N ratio) and plant uptake, and consequently reduced the content of mineral and extractable N in the soil. The high losses of unlabeled N by leaching, compared to the low losses of labeled N can be explained by the apparent rapid gaseous losses of the added mineral N or its transformation to microbial biomass or organic forms.

The cumulated drainage at 30 cm depth was less than 35 mm, which represented 8% of rainfall and irrigation together. Nevertheless, this low drainage generated important losses by leaching. The same tendency was found by Peralta and Stockle (2002) in a simulation study where the conclusion was that, rather than a moderate excess of irrigation, fertilization is the main cause of leaching. As drainage is difficult to avoid, due to the unpredictable nature of rainfall, and as small drainage rates produced high rates of leaching, it is important to reduce the leaching potential by controlling fertilization (type, timing and amount) and irrigation. In this experiment, it was the addition of rice hulls that seemed to be more effective in controlling this kind of loss.

Considering MF as the control treatment, where all N was applied in mineral form, and considering the different effects of the organic substrates on plant N uptake and losses, the results indicate that in this soil, with its high content of potentially available N, the application of rice hulls improved the use efficiency of the N applied in mineral form and of total N, increasing crop yield and decreasing leaching losses. However, this substrate was less effective in controlling gaseous losses of the mineral fertilizer. On the other hand, the application of poultry manure did not significantly improve NUEf and tuber yield, nor did it prevent leaching losses, but it seems to have significantly decreased gaseous losses from the mineral fertilizer. In the combined treatment (P + R + F), NUEf and tuber yield increased, whereas gaseous and leaching losses decreased, compared to MF, as a result of the differential effect of each organic substrate on soil processes. Despite the slightly lower yield in the combined treatment compared to R + F, the indices of use/leaching and use/total losses were higher, indicating the advantages of this substrate combination from an environmental point of view.

The initial soil N contributed significantly to the crop's N nutrition in all cases. Subsequently, it can be assumed that the effect of the treatments on N uptake and tuber yield will be different in soils that have received lower doses of poultry manure and other sources of N in the past and consequently have lower levels of potentially available N.

Manipulating the quality and quantity of organic and mineral amendments can improve the synchronization between N availability and crop requirements and therefore increase NUE and reduce N losses. However, the effect of each substrate depends on initial soil N availability, and on soil and climate conditions, which determine the predominance of one or another soil process (e.g. soil acidity is the main control of volatilization whereas rainfall, irrigation and soil texture are the main factors controlling leaching). In this study, where soil pH was near to neutral, due to the application of lime in previous crop cycles, gaseous losses may have been favored. In this context, it should be acknowledged that the development of strategies to limit N losses from one route may exacerbate losses from another (Chambers et al. 2000). Consequently, the same treatment can produce different results depending on soil and environmental conditions, making it difficult to generalize the results of a particular experiment (Salazar et al. 2005). A detailed knowledge of the influence of each substrate on soil processes under different conditions is required to design appropriate management strategies based on the combination of different sources of N.

Conclusions

The application of: (1) the substrate of low C/N (poultry manure), improved the synchronization between N availability in the soil and crop requirements, because it promoted the fast transformation of the applied mineral N into organic forms (microbial biomass and soil extractable organic matter), avoiding losses when crop requirements were low; (2) the substrate of high C/N (rice hulls), improved the N plant uptake, probably by stimulating root growth; and (3) the substrate combination improved both aspects and allowed to obtain a high yield with lower N losses and a higher index of use/losses.

Acknowledgments This research was founded by FONACIT (grants S1-2000000810 and F-2002000424) and framed within the MOSANDES project (CYTED, project XII.4). We wish to thank Francis Guillén, Zulay Mendez, Sonia Morales, Nelson Marquez and Alexander Suarez for their technical assistance, Carlos Díaz for his support during fieldwork and the fruitful discussions, Gloria and Ana Graziella Machado for all their kind cooperation and Julia K. Smith for the English revision.

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