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Influence of redox conditions and rice straw incorporation on nitrogen availability in fertilized paddy soils

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Abstract

Temporal nitrogen (N) availability in fertilized rice paddies is the result of a balance of processes, mainly the gross rates of N mineralization, microbial and abiotic immobilization and N losses. Water and crop residue management practices often confound these established relationships making N the most difficult nutrient to manage in rice cropping systems. To investigate and quantify the interactive effects of soil redox conditions and straw incorporation on temporal fertilizer-N availability we treated a paddy soil with enriched ammonium-¹⁵N and incubated for 160 days under flooded or non-flooded conditions, with or without the addition of rice straw. Changes in total available N as well as available and immobilized fertilizer-derived N (FDN) during incubation were evaluated. Under both oxic and waterlogged soils about 45–53% of applied N was rapidly immobilized. Whereas in the former most of this FDN was released contributing to the available N pool, flooded soils experienced significant losses from the soil/water system (\approx 67% of applied N). Addition of rice straw enhanced N immobilization, particularly under flooded conditions, that also contributed to limiting losses. Moreover, turnover of this labile organic matter pool supplied significant amounts of available N towards the later stages of the incubation, partly compensating for the immobilization of fertilizer-N.

Keywords: Fertilizer-derived nitrogen; indigenous nitrogen supply; immobilization; temporal variations; clay mineralogy; ¹⁵N stable isotope tracing.

Introduction

Rice is the most important food crop of the world's population with 159 Mha under cultivation globally (FAO, 2008) principally in tropical but also in temperate zones. Rice is predominantly grown in flooded paddies that lead to anoxic soil conditions over a major part of the cropping period. Redox processes in paddy soils thus play an important role in controlling nutrient availability, element cycling, and ecological functions of rice agroecosystems (Yu et al. 2007).

In particular, nitrogen (N) is the most complex and yield-limiting nutrient to manage in rice cropping systems worldwide (Reddy 1982; Eagle et al. 2000). Although N supply drives productivity (Mikkelsen 1987; Cassman et al. 1996; Mae 1997), poor N fertilizer-use efficiency (30–40% recovery of applied N) is characteristic of irrigated rice systems (Cassman et al. 2002). Several field studies have shown that only *ca.* 37% of total N uptake by rice is supplied by fertilizer-N, with the remainder derived from native soil N (Reddy 1982; Cassman et al. 1998). Mineral fertilizers may promote the mineralization of soil organic N (Devêvre and Horwáth, 2000) with strong short-term changes in the soil organic matter (SOM) turnover, which in turn affects plant N availability at different extent during the crop cycle. The

incorporation of crop residues further influences the processes that control N availability in rice paddies (Bird et al. 2003). The simultaneous addition of N fertilizers and crop residue incorporation may lead to significant microbial N immobilization during the decomposition of labile straw-derived OM (Fontaine and Barot 2005; Moorhead and Sinsabaugh 2006). Nitrogen immobilized by the soil microbial biomass may be subsequently remineralized or redistributed among more complex heterogeneous and less labile fractions of SOM (Devêvre and Horwáth 2001; Nannipieri and Eldor 2009), although it is poorly known how these processes affect temporal variation of N availability during the crop season (Saharwat 2005). Crop residue incorporation may further contribute to N immobilization via abiotic reactions of ammonia with SOM (Olk et al. 2006), which can reduce both N concentration in soil solution and feedback to microbial utilization. As a result both positive (Sharma and Mittra 1988; Mahapatra et al. 1991; Alberto et al. 1996; Cassman et al. 1996) and negative (Rao and Mikkelsen 1976; Beri et al. 1995) effects of crop residue incorporation on N availability and rice yields have been observed. Moreover, in soils containing appreciable amounts of 2:1 clay minerals, N availability may be further limited by the capability of these layer silicates to rapidly fix important amounts of ammonium in their interlayer (Wang et al. 2001; Nieder et al. 2011).

Besides immobilization, the combination of crop residue and flood-water management practices may strongly influence N losses from paddy fields to the atmosphere or adjacent water bodies, that together may represent between 10–65% of applied fertilizer-N (Cassman et al. 1998; Ghosh and Bhat 1998), with important implications on greenhouse gas emissions and water quality.

Based on these considerations N immobilization and losses are not only responsible for an overall limited efficiency of applied fertilizer, but also for a high temporal variability in N availability during the cropping season as a function of water and crop residue management. Although several studies have evaluated the processes controlling N availability in rice fields, our knowledge on how the simultaneous addition of fertilizer-N and rice straw affects temporal changes in N availability under water saturated conditions is far from complete (Saharwat 2005). We therefore hypothesised that (i) redox conditions influence the temporal variations in N availability as a function of different interrelated processes which control the distribution of fertilizer N among available, immobilized and lost pools, and (ii) although the simultaneous addition of fertilizer-N and rice straw may lead to the immobilization of important amounts of applied N, this may contribute to reducing N losses and increasing OM turnover under flooded conditions thus resulting in a net increase in available N with time. We tested these hypotheses by investigating and quantifying the interactive effects of soil redox conditions and straw incorporation on temporal variations in N availability in a fertilized paddy soil over 160 days, corresponding to one rice crop season in temperate agroecosystems. We adopted ¹⁵N-stable isotope tracing to distinguish between fertilizer-derived and straw-derived or indigenous N and to evaluate the distribution of applied N among available, immobilized and lost N pools.

Materials and methods

Soil and plant materials

Soil was collected from the Ap horizon of a paddy field under continuous rice cultivation for the last 30 years, situated within a long-term experimental platform in Vercelli, NW Italy (45°17'47"N, 8°25'51"E). The soil is a *Typic Endoaquept* (Soil Survey Staff 2010) or *Haplic Gleysol* (FAO 1998). In addition, topsoil volume samples (100 cm³ cores) were taken for bulk density determination. After removal of vegetation and visible roots, the bulk soil was homogenized by passing through a 2 mm sieve without drying and stored fresh until use. An aliquot of the soil was air-dried and sieved to < 2 or < 0.5 mm for physical and chemical

characterization. Rice straw (*Oryza sativa* variety Sirio CL) was sampled after grain harvest from the same paddy field. The straw was chopped into pieces of approximately 1 cm in length and subsequently dried at 40°C. A portion of the straw was homogenized to < 0.5 mm for chemical analysis.

General characterization

The bulk density of the soil was determined by weighing the 100 cm³ soil cores after drying at 105°C, while the water holding capacity was determined gravimetrically. The pH of the bulk soil was measured potentiometrically in H₂O at a soil-to-solution ratio of 1:2.5. Total C and N contents of the bulk soil and rice straw were measured on ground sub-samples (< 0.5 mm) by dry combustion (NA2100, CE Instruments, Milan, Italy). Since the soil material was free of carbonate the total C was considered as organic C. Soil inorganic N content was determined spectrophotometrically as described below. The cation exchange capacity was determined using 0.1 M BaCl₂ at pH 8.1 (Sumner and Miller, 1996) and the exchangeable amounts of Ca, Mg and K measured by atomic absorption spectrometry (Perkin Elmer AAnalyst 1400, USA).

Particle-size of the soil material (< 2 mm) was measured by the sieve-pipette method after dispersion of the sample with sodium hexametaphosphate (Gee and Bauder 1986), without destruction of oxides and OM. Mineralogical characterization of the clay fraction (< 2 µm) separated by sedimentation was carried out by X-ray diffraction (Philips PW-1710, Almelo, The Netherlands). Oriented mounts of (i) Mg-saturated, (ii) ethylene glycol solvated, and (iii) heated (550°C) clay samples were scanned from 3-35°2θ with Co-Kα radiation (40 kV; 20 mA) at a speed of 1.5°2θ min⁻¹. The identification of clay minerals was made by evaluating the variations in peak positions and intensities in all XRD patterns according to Thorez (1975). Aluminum and Fe from amorphous oxides and bound to OM were extracted from bulk soil samples with 0.2 M NH₄-oxalate (pH 3) by the method of Schwertmann (1964). Iron in amorphous and crystalline oxides was estimated by the dithionite–citrate–bicarbonate method (Mehra and Jackson 1960). Aluminum and Fe in the extracts were measured by atomic absorption spectrometry (Perkin Elmer AAnalyst 1400).

Soil incubation

To evaluate the influence of redox soil conditions and rice straw addition on the availability of applied N, the collected paddy soil was incubated under (i) non-flooded (NF) or flooded (F) conditions, (ii) without (NS) or with (S) the addition of rice straw, in a completely randomized 2×2 factorial arrangement. For each treatment a series of 30 plastic containers were filled with 200 g of fresh, homogenized soil and packed to field bulk density. Non-flooded soils were kept at 50% of their water holding capacity by adjusting the water content every alternate day to correct for any soil moisture lost through evaporation. Flooded soils were submerged under 5 cm of degassed and deionized water. Before packing, soils receiving straw were added with 860 mg of rice straw (4.3 g kg⁻¹ soil d.w, equivalent to a field application dose of 10 Mg ha⁻¹) and thoroughly mixed. After a 7 day pre-incubation period, inorganic N was added to all soils as (NH₄)₂SO₄ at an application dose of 56 mg N kg⁻¹ equivalent to 130 kg N ha⁻¹ generally applied as mineral fertilizer in the field. To half of the samples inorganic N was added as enriched (NH₄)₂SO₄ (10 at% ¹⁵N), while the other half was treated with natural abundance (NH₄)₂SO₄ to account for isotope fractionation. Incubation was carried out for 160 d at 25°C in the dark under non-leached conditions. Soil pH and Eh were measured potentiometrically in triplicate over the whole incubation period in a separate set of samples. After 1, 10, 30, 90 and 160 d from N fertilization, three containers for each treatment were destructively sampled and the soils immediately frozen until analysis. Floodwaters in submerged samples were however previously carefully decanted into measuring cylinders

taking care not to lose any suspended material, total volume recorded, and subsequently filtered at 0.45 µm and frozen until analysis.

Inorganic N, and dissolved organic C and N

Inorganic N was extracted from moist soil samples in 0.5 M K₂SO₄ (solid to water ratio of 1:4 w/v), suspensions centrifuged at 1,200×g for 10 min, and the supernatant filtered first through Whatman No. 42 filter paper and subsequently through a 0.45 µm membrane filter. Moisture content was also determined on a separate aliquot of soil to allow for expressing all concentrations on a dry soil weight basis. Ammonium (NH₄⁺) concentrations in soil extracts and floodwaters were determined spectrophotometrically by a modified Berthelot method involving reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson 1971). Nitrites (NO₂⁻) were determined spectrophotometrically by utilizing a modified Griess reaction involving the formation of an azo-dye by reaction of NO₂⁻ with sulfanilamide under acidic conditions, and subsequent complexation of the diazo compound formed with N-(1-naphthyl)-ethylenediamine dihydrochloride (Mulvaney 1996). Simultaneous determination of NO₂⁻ and nitrates (NO₃⁻) was carried out by quantitatively reducing NO₃⁻ to NO₂⁻ by addition of VCl₃ in the presence of Griess reagents (Miranda et al. 2001) and heating at 40°C for 3 h. Dissolved organic C (DOC) and total bound N (TN_b) were determined simultaneously in acidified (pH=2) aliquots of soil extracts and floodwaters by Pt-catalyzed, high-temperature combustion (680°C) followed by infrared and electrochemical detection of CO₂ and NO, respectively (Vario TOC, Elementar, Hanau, Germany). Dissolved organic N (DON) was calculated as the difference between TN_b and the sum of all inorganic N forms.

Fertilizer-derived N in soil extracts and floodwaters

The fraction of inorganic N present in soil extracts and floodwater samples derived from applied N was considered to represent available fertilizer-derived N (FDN). This was determined by a combination of micro-diffusion and ¹⁵N stable isotope analysis as described by Schleppi et al. (2006). Briefly, an aliquot of soil extract or floodwater containing about 80 µg N was transferred into a 100 ml glass vial together with a teflon-coated, citric acid trap. After addition of 0.4 g Devarda's alloy for NO₃⁻ and NO₂⁻-N reduction, and 0.2 g MgO for NH₃ volatilization, the vials were immediately sealed with a crimp-top septum, gently shaken, and incubated for 7 days at 25°C to allow for complete diffusion of NH₃ onto the acid trap. The isotope ratio of trapped N was subsequently measured by elemental analysis coupled to a continuous flow isotope ratio mass spectrometer (FlashEA-1112 interfaced via a ConFlo III to a Delta Plus XP, Thermo Finnigan, Bremen, Germany). Fertilizer-derived N (FDN) was calculated from blank-corrected ¹⁵N isotope ratios (Stark and Hart 1996) of trapped N according to the equation:

$$FDN = \left[\frac{at\%^{15}N_{\text{sample}^{15}N\text{-soil}} - at\%^{15}N_{\text{sample}^{14}N\text{-soil}}}{at\%^{15}N_{^{15}N\text{-fertilizer}} - at\%^{15}N_{^{14}N\text{-fertilizer}}} \right] \cdot N_{\text{sample}}$$

where, at%¹⁵N_{sample} is the isotope ratio of soil extracts or floodwater samples obtained from soils (¹⁵N-soil or ¹⁴N-soil) receiving enriched or natural abundance N fertilizer (¹⁵N-fertilizer or ¹⁴N-fertilizer), respectively, and N_{sample} is the total inorganic N content of that sample.

Immobilized fertilizer-derived N

After extracting soil samples with 0.5 M K₂SO₄, the residue was washed repeatedly with deionized water until the electrical conductivity of the supernatant was <50 µS cm⁻¹. After

each centrifugation step the supernatant was aspirated with a fine needle taking care not to lose any suspended organic material. Washed samples were then dried, crushed to <0.5 mm and analyzed for total N and isotopic ratio. The amount of immobilized fertilizer-N was calculated using the above equation.

Statistical analysis

All results were reported or plotted as the mean of six (inorganic N forms, and DOC) or three (available or immobilized FDN) replicates. The effects of redox conditions, straw addition and incubation time as well as their interactions on available N forms, available and immobilized FDN and DOC concentrations were tested by three-way analysis of variance (ANOVA), while two-way ANOVA was used to test the effects of these variables and their interactions on inorganic N concentrations in floodwaters. Least significant difference at $P = 0.05$ was used for comparing means.

Results

Soil and straw characteristics

The neutral topsoil (pH = 6.3) used in this study had a field bulk density of 1.55 g cm^{-3} , a saturated water holding capacity of 405 g kg^{-1} , a sandy loam texture (6.5% clay and 41.1% silt) and relatively low total C and N contents (11.6 and 1.1 g kg^{-1} , respectively). Ammonium and nitrate-N contents were 3.3 and 5.5 mg N kg^{-1} respectively, whereas nitrites were not detected. Low SOM and clay contents resulted in relatively low cation exchange capacity ($6.7 \text{ cmol}_{(+)} \text{ kg}^{-1}$) and exchangeable cations (0.19 , 5.4 and $1.0 \text{ cmol}_{(+)} \text{ kg}^{-1}$ for K^+ , Ca^{2+} and Mg^{2+} , respectively).

The results of XRD analyses on the clay fraction are reported in Fig. 1. The diffractogram of Mg-saturated clay showed diffuse bands centred at 1.41 , 1.00 , 0.70 , 0.50 , 0.47 , 0.35 and 0.33 nm . The presence of illite was deduced by the characteristic reflections at around 1.00 , 0.50 and 0.33 nm in both ethylene glycol solvated and heat treated clay samples, while the relatively low intensity of the 1.41 , 0.70 and 0.35 nm reflections and the increase in intensity of the reflection at around 1.00 nm in the heat treated sample suggests the presence of vermiculite. In the latter diffractogram, the low intensity reflection at 1.41 nm and the broad band centred at 1.18 nm were due to chlorite and chlorite-vermiculite mixed-layer phases, while the similarity in Mg-saturated and ethylene glycol diffractograms suggests that smectite minerals were absent. Moreover, the soil had relatively low contents of oxalate-extractable Fe and Al (3.2 and 0.6 g kg^{-1} , respectively), and dithionite-citrate-bicarbonate extractable Fe (7.0 g kg^{-1}).

The total C and N contents of the straw were 400 g C kg^{-1} and 6.6 g N kg^{-1} , respectively.

Redox potential and pH

Variations in redox potential and pH during incubation for each treatment are presented in Fig. 2. Under non-flooded conditions redox potentials remained relatively constant throughout the incubation, both in the presence (NF-S) or absence (NF-NS) of straw. In these treatments Eh values were always positive with a mean value of $+511 \pm 6 \text{ mV}$. In contrast, under flooded conditions Eh values tended to decrease rapidly during the early stages of incubation as a result of the onset of anaerobic conditions. In the presence of straw (F-S) this drop in Eh was faster and lower potentials were reached with respect to flooded soils not receiving straw (F-NS). In fact, in the former Eh dropped from $+293$ to -180 mV over the first 30 d of incubation, while in the latter Eh decreased from $+351$ to $+110 \text{ mV}$ over the same period, and did not decrease below $+9 \text{ mV}$ over the whole incubation. During the later stages of incubation, redox potentials in both F-S and F-NS soils were similar and relatively constant ($\text{Eh} \approx +30 \text{ mV}$). pH values of non-flooded samples were rather constant during the incubation

with mean pH values of 5.2 ± 0.1 and 4.9 ± 0.1 for soils with (NF-S) and without (NF-NS) straw, respectively. On the other hand, pH values of flooded soils with or without straw tended to increase towards a pH of 7.0. This increase was nonetheless faster in soils receiving straw, particularly during the early stages of incubation.

Inorganic N dynamics

The effects of soil redox conditions, addition of rice straw, incubation time as well as their interactions, on total NH_4^+ and NO_3^- concentrations during incubation were significant ($p < 0.001$). Figure 3 shows the variations in the concentrations of inorganic N forms, expressed as the sum of NH_4^+ or NO_3^- in K_2SO_4 soil extracts and floodwaters (in flooded soils), with incubation time as a function of soil redox conditions and straw incorporation. In all soil samples, NO_2^- concentrations were always below the limits of detection. Under non-flooded conditions rapid nitrification of the added fertilizer led to a rapid decrease in NH_4^+ and a simultaneous increase in NO_3^- concentrations within the first 30 d of incubation. However, after 30 d the samples to which straw was added (NF-S) showed lower NO_3^- concentrations ($42\text{--}55 \text{ mg N kg}^{-1}$) with respect to samples not receiving straw (NF-NS; $60\text{--}78 \text{ mg N kg}^{-1}$; $p < 0.001$). In contrast, under flooded conditions NH_4^+ was the principal inorganic N form, as all NO_3^- originally present in the soils was rapidly lost (Fig. 3). With time NH_4^+ concentrations tended to decrease in the absence (F-NS) and increase in the presence (F-S) of straw ($p < 0.001$). In the latter, after 90 d of incubation NH_4^+ concentrations were equal or greater than applied fertilizer-N.

Table 1 reports the variations in the concentration of inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) in floodwater samples with incubation time for flooded soils without or with straw. The effects of straw addition, incubation time and their interaction were significant ($p < 0.001$). On average, 5–26% of the total available N was found in floodwaters in both treatments with highest concentrations ranging between $13\text{--}14 \text{ mg N kg}^{-1}$, mainly as NH_4^+ (data not shown). Changes in floodwater inorganic N concentrations with incubation followed similar trends as those observed for total NH_4^+ . In fact, floodwater N concentrations in F-S were initially similar (first 30 d of incubation) but subsequently higher with respect to F-NS samples.

Figure 4 shows the variations in total available N (top line) expressed as the sum of NH_4^+ and NO_3^- in K_2SO_4 soil extracts and floodwaters (in flooded soils), with incubation time as a function of soil redox conditions and straw incorporation. Even here, the effects of redox conditions, straw addition, incubation time and their interactions were all significant ($p < 0.001$). In both non-flooded and flooded soils, straw application resulted in lower initial available N contents with respect to soils not receiving straw ($p < 0.05$). However, whereas in non-flooded soils total available N was generally lower in soils receiving straw ($39\text{--}60 \text{ mg N kg}^{-1}$) with respect to those without straw ($53\text{--}79 \text{ mg N kg}^{-1}$), the opposite was true for flooded soils particularly during the later stages of the incubation ($p < 0.001$). In fact, total available N over the incubation period ranged between $21\text{--}57$ and $39\text{--}72 \text{ mg N kg}^{-1}$ for F-NS and F-S samples, respectively.

Fertilizer-derived N dynamics

^{15}N stable isotope analysis of soil extracts, floodwaters and K_2SO_4 extracted soils allowed for the quantification of available and immobilized FDN. Under non-flooded conditions, 45–53% of applied N was rapidly immobilized after 1 d of incubation resulting in an important reduction in available FDN (Fig. 4; Table 2). A major part of this immobilized FDN was released within the first 10 d of incubation resulting in a relative increase in available FDN reaching values around 45 mg N kg^{-1} . Subsequently, available FDN tended to decrease with time reaching values between $20\text{--}24 \text{ mg N kg}^{-1}$ towards the end of the incubation, equivalent to 36 and 43% of applied N in the presence and absence of straw, respectively. Apart from a

slightly higher FDN availability in the absence of straw, immobilized FDN was also generally lower with respect to soils receiving straw ($p < 0.01$). In fact, on average 9 and 23% of applied N (excluding the initial immobilization on day 1) was immobilized in the no straw and straw treatments, respectively. By the end of the incubation period 42–49% of fertilizer N applied to non-flooded soils was neither present in available or immobilized N pools.

As for non-flooded soils, around 46% of applied N was rapidly immobilized within 1 d of incubation under flooded conditions, both in the absence and presence of straw. This resulted in initial available FDN contents of around 30 mg N kg⁻¹, equivalent to 53% of the applied N. However, in contrast to non-flooded soils, immobilized FDN contents either decreased slowly or remained relatively constant with time for no straw and straw treatments, respectively. Notwithstanding the release of immobilized FDN during the incubation of flooded soils without straw, this treatment showed the lowest available FDN contents with values as low as 3 mg N kg⁻¹ equivalent to only 6% of the applied N observed towards the end of the incubation. In fact, this treatment also showed the highest difference between applied N and the sum of immobilized and available FDN (67% unaccounted FDN). On the other hand, flooded soils receiving straw showed a greater mean immobilization of FDN (26 mg N kg⁻¹, equivalent to 47% of the applied N) and available FDN contents with respect to flooded soils not receiving straw. This resulted in the lowest amounts of unaccounted FDN ranging between 1 and 24% of applied N. Between 6 and 47% of the available FDN in flooded soils was present in floodwaters (Table 1). Highest values of FDN in floodwaters equivalent to about 79% of applied N were measured after 10 d of incubation in both straw and no straw treatments (Table 1). In fact, FDN constituted between 82 and 16% of the inorganic N present in floodwaters, with a significantly higher percentage contribution of FDN towards the beginning of the incubation ($p < 0.01$) that tended to decrease with time in both treatments.

Dissolved organic carbon and nitrogen

Redox conditions, straw addition and incubation time significantly influenced DOC concentrations ($p < 0.05$) while the interaction among variables was not significant. Dissolved organic C concentrations were generally relatively high towards the beginning of the incubation with values around 195–263 mg C kg⁻¹ (Fig. 5). Within 10 d of incubation DOC contents decreased rapidly to 56–69 mg C kg⁻¹ across all treatments ($p < 0.001$). However, whereas DOC content subsequently remains relatively low throughout the incubation (mean contents of 65 mg C kg⁻¹) under non-flooded soil conditions, it increased progressively with time under flooded conditions. This increase was enhanced in the presence of straw ($p < 0.001$) resulting in final DOC contents of 155 and 116 mg C kg⁻¹ for F-S and F-NS, respectively. Dissolved N was predominantly constituted by inorganic N forms resulting in negligible or non-quantifiable DON concentrations throughout the incubation (data not shown).

Discussion

Results evidence how both soil redox conditions and addition of rice straw may strongly influence N dynamics in fertilized rice paddies with important implications on temporal N availability. It is generally assumed that net ammonification and consequently N availability are greater under anaerobic soil conditions due to the low metabolic N requirements of anaerobic microorganisms (Reddy and DeLaune 2008). However, the higher N availability found in non-flooded with respect to flooded soils as well as the temporal dynamics in N availability question this assumption.

Under our experimental conditions, N availability was initially affected by a rapid and intense soil immobilization, independent of the redox conditions or straw addition. This led to the retention of 45–53% of the applied N within 1 d of incubation. Many studies have documented a rapid immobilization of applied NH₄⁺ into non-exchangeable pools in both

upland (e.g. Compton and Boone 2002; Morier et al. 2008) and lowland (e.g. Green et al. 1994; Zhang and Scherer 2000) soils. The considerable presence of vermiculite and interstratified chlorite-vermiculite characterizing the mineralogy of the soil under study, as well as the low percentage of K^+ and NH_4^+ saturation, suggest that interlayer NH_4^+ fixation represents a key process determining fast N immobilization (Said-Pullicino et al. 2013).

Under aerobic conditions a large part of immobilized N was released to the exchangeable and soluble phase within the first 10 d of incubation and rapidly converted into nitrate. This release was assumed to be diffusion controlled (Matsuoka and Moritsuka 2011; Nieder et al. 2011; Said-Pullicino et al. 2013) and governed by the decreasing concentration of NH_4^+ in the soil solution, which disappeared completely after 30 d of incubation, due to nitrification (Green et al. 1994). The addition of ammonium fertilizer to soil might have also stimulated the growth of nitrifying bacteria favouring nitrification activity (Hastings et al. 1997; Chu et al. 2007). Isotopic data confirmed that the release of immobilized FDN was mainly responsible for the increase in available N in the first days of incubation (Fig. 4), as indigenous N supply over this period was relatively constant. However, in the presence of straw more FDN was retained resulting in the immobilization of 23% of applied N by the end of the incubation with respect to 8% in aerobic soils without straw. The addition of rice straw (C/N = 61) could have supplied labile organic C to heterotrophic microorganisms leading to an increase in the biotic immobilization of both FDN and non-FDN available pools. This could have been responsible for the lower available N contents observed in soils receiving straw with respect to those without straw, for both FDN and non-FDN pools. The presence of readily oxidizable organic compounds might have also stimulated competition between heterotrophic microorganisms and nitrifying bacteria for O_2 and available nutrients leading to a reduction in nitrification rates (Prosser 1989) thereby influencing the equilibrium between exchangeable and fixed NH_4^+ . Notwithstanding the enhanced immobilization in the presence of straw, the aerobic degradation of added labile OM favoured the release of indigenous and straw-derived inorganic N forms (Robertson and Groffman 2007) resulting in an increase in the proportion of available N in the non-FDN pool from 24 to 60% with incubation.

In contrast to aerobic soils, the release of immobilized N under water saturated conditions was rather limited. Devêvre and Horwáth (2001) found a higher and sustained microbial biomass under flooded conditions that can immobilize more N and make it less available for plant uptake. We can also infer that the inhibition of nitrification under anaerobic conditions, responsible for greater exchangeable NH_4^+ concentrations (Fig. 3), determined a slower release of weakly fixed FDN from the interlayer of clay minerals compared to oxic conditions (Said-Pullicino et al. 2013). In addition, the reductive dissolution of Fe (hydr)oxides coated on the surface of clay minerals could have favoured the diffusion and fixation of NH_4^+ ions into the interlayers, thereby enhancing retention (Scherer and Zhang 1999; Sahrawat 2004; Nieder et al. 2011). These biotic and abiotic immobilization processes limited N availability which, over the first 10 d of incubation, was 29% less with respect to oxic conditions without straw. However, other processes could have accounted for the drastic decline in N availability observed after 30 d of incubation. The prevailing coupled processes of simultaneous nitrification and denitrification and the anaerobic ammonium (NH_4^+) oxidation may be responsible for the loss of an appreciable part of both native and applied N in flooded rice soils (Sahrawat 1980; Buresh et al. 2008; Zhu et al. 2011). Moreover, the appreciable concentrations of NH_4^+ in the floodwaters may have favoured NH_3 volatilization (De Datta 1995), although these losses are generally more important within the first days after N application (Buresh et al. 2008). As observed for non-flooded soils, the amount of indigenous available N did not vary considerably during anoxic incubation, notwithstanding the significant release of dissolved organic matter resulting from the reductive dissolution of Fe(hydr)oxides, increase in pH and/or SOM decomposition and mineralization. As a result this

treatment showed the highest amount of unaccounted FDN (up to 67% of total applied N) and the lowest fertilizer-N availability (6–10% towards the end of the incubation), of which a substantial proportion was partitioned in the flood water, further increasing the potential losses and decreasing plant availability.

Addition of rice straw to flooded soils resulted in the greatest immobilization of applied N (44–50%) with respect to the other treatments, throughout the incubation period. This could have been due to a combination of biotic and abiotic factors. Addition of a relatively labile C source could have enhanced microbial immobilization of added N (Bird et al. 2001; Burger and Jackson 2003; Peng et al. 2009). Moreover, the increase in exchangeable NH_4^+ resulting from the inhibition of nitrification under anaerobic conditions in combination with the mineralization of added labile OM (Fig. 3) could have limited the diffusion and release of fertilizer-derived NH_4^+ initially fixed in the clay mineral interlayers. The enhanced reductive dissolution of Fe (hydr)oxides at low Eh values (~ 180 mV) and pH values around 7 (Kato et al. 2005) further promoted NH_4^+ fixation (Matsuoka and Moritsuka 2011). These immobilization processes contributed to reducing available FDN contents that decreased from 53 to 27% of applied N over the incubation period. However, in contrast to flooded soils without straw addition, only 24% of applied N was unaccounted by the end of the incubation, suggesting that addition of straw strongly limited N losses. In addition, the reduced availability of FDN during the first days of incubation was compensated by a strong increase in non-FDN availability (both soluble and exchangeable) particularly during the later stages of incubation. The fast turnover of OM in these systems could rapidly result in a shift from net N immobilization to net N mineralization, particularly under anaerobic conditions characterized by a microbial biomass with low N requirements. The non-FDN available pool was probably fed by the mineralization of labile straw compounds and SOM coupled with an ongoing release of dissolved OM following reductive dissolution of Fe(hydr)oxides at Eh ≈ 30 mV and neutral pH. The negligible amounts of dissolved organic N determined are in line with a rapid turnover of this labile N pool and the consequent increase in inorganic available N forms (Bird et al. 2001; Sahrawat 2006; Nannipieri and Eldor 2009; Kögel-Knabner et al. 2010). These results are in contrast with the negative effects of straw incorporation on N uptake and rice yield observed in some studies (e.g. Beri et al. 1995; Bird et al. 2001), suggesting that the benefits of crop residue incorporation in reducing N losses and enhancing N availability for plant uptake depend on the synchrony between these processes and the physiological N requirements of the crop. In fact, these benefits could be drastically outweighed if the release of immobilized fertilizer-N or mineralization of incorporated crop residues occurs in correspondence with the later stages of the cropping cycle (e.g. crop maturity) or during the subsequent intercrop fallow period. However, we cannot exclude that the release of immobilized fertilizer-N was somewhat underestimated with respect to field conditions where the interaction between plant uptake, microbial diversity and soil properties (Chaparro et al. 2012) would further influence this process. Indeed, Bird et al. (2001) found that the lower recovery of FDN in the crop from residue incorporated plots was balanced by an increased N recovery in the soil in the spring of the following year and a small recovery of applied N in grain of subsequent crop.

Conclusions

Temporal variations in available N in fertilized paddy soils are strongly regulated by complex and interactive mechanisms induced by soil redox conditions and crop residue incorporation. Soil flooding generally increased the amount of fertilizer-N immobilized onto the soil, accompanied by a strong decline in both fertilizer-derived and indigenous available N forms with respect to non-flooded soils. The incorporation of rice straw in flooded soils provided an easily mineralizable source of labile OM for microbial utilization over time, which both

enhanced fertilizer-N immobilization reducing N losses, and supplied important amounts of straw-derived available N with time. Although N availability in fertilized paddy soils are strongly coupled to crop residue and flood water management practices, synchrony between fertilizer and indigenous N supply, and plant uptake is thus a crucial factor in determining fertilizer use efficiency and crop yield.

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Table 1: Variations in floodwater inorganic N contents with time for flooded soils without or with straw addition.

Incubation time (d)	Total N ^a (mg N kg ⁻¹)	Fertilizer-derived N ^b	
		(mg N kg ⁻¹)	(% of total)
<i>Flooded, without straw</i>			
1 d	5.7 ± 0.7	4.7 ± 0.6	82
10 d	13.4 ± 0.4	10.6 ± 0.3	79
30 d	10.1 ± 0.8	7.1 ± 0.5	70
90 d	1.8 ± 0.2	0.5 ± 0.1	26
160 d	1.2 ± 0.5	0.2 ± 0.1	16
<i>Flooded, with straw</i>			
1 d	3.0 ± 0.5	2.4 ± 0.4	81
10 d	13.5 ± 0.5	10.8 ± 0.4	80
30 d	8.7 ± 0.8	5.1 ± 0.5	59
90 d	13.9 ± 1.6	4.2 ± 0.5	30
160 d	11.7 ± 0.8	2.7 ± 0.4	23

^a Calculated as the sum of ammonium and nitrate-N concentrations;

^b Calculated from the isotope enrichment of inorganic N in the flood-waters.

Table 2: Variations in the percentage distribution of applied fertilizer-N between available and immobilized pools with time for soils incubated under non-flooded or flooded conditions, without or with straw addition.

Incubation time (d)	Available (%)	Immobilized (%)	Unaccounted ^a (%)
<i>Non-flooded, without straw</i>			
1 d	37	53	10
10 d	83	12	5
30 d	67	9	23
90 d	64	8	28
160 d	43	8	49
<i>Non-flooded, with straw</i>			
1 d	54	45	1
10 d	75	25	0
30 d	48	24	28
90 d	43	21	35
160 d	36	23	42
<i>Flooded, without straw</i>			
1 d	54	46	0
10 d	50	36	14
30 d	62	36	3
90 d	10	29	60
160 d	6	27	67
<i>Flooded, with straw</i>			
1 d	53	46	1
10 d	41	44	15
30 d	40	46	14
90 d	29	50	21
160 d	27	49	24

^a Calculated from the difference between applied fertilizer-N, and the sum of available and immobilized FDN.

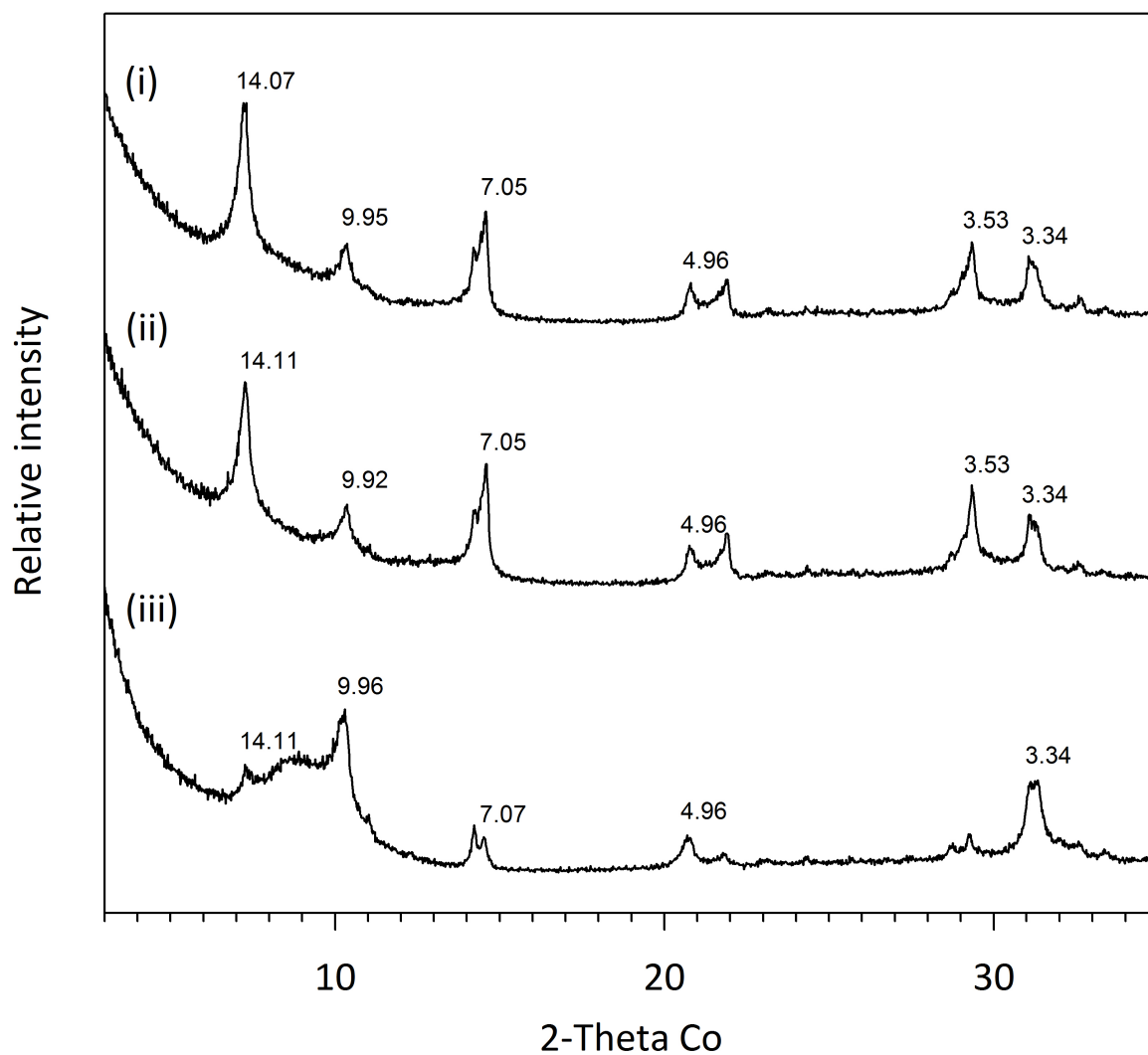


Figure 1: X-ray diffractograms (Co K α radiation) of (i) Mg-saturated, (ii) ethylene glycol solvated, and (iii) heated (550°C) clay samples. Labels represent basal spacing (nm).

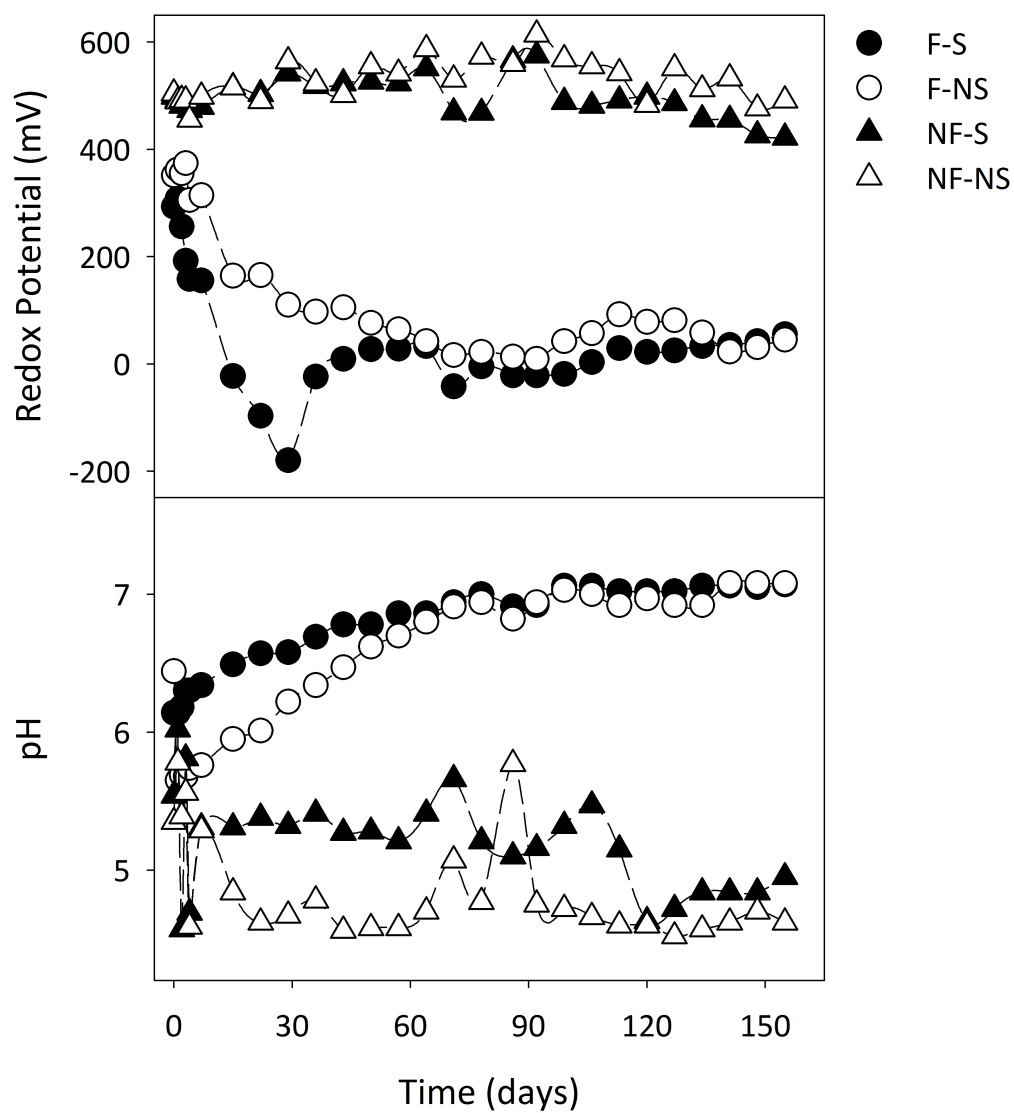


Figure 2: Variations in soil redox potential and pH with time for soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition.

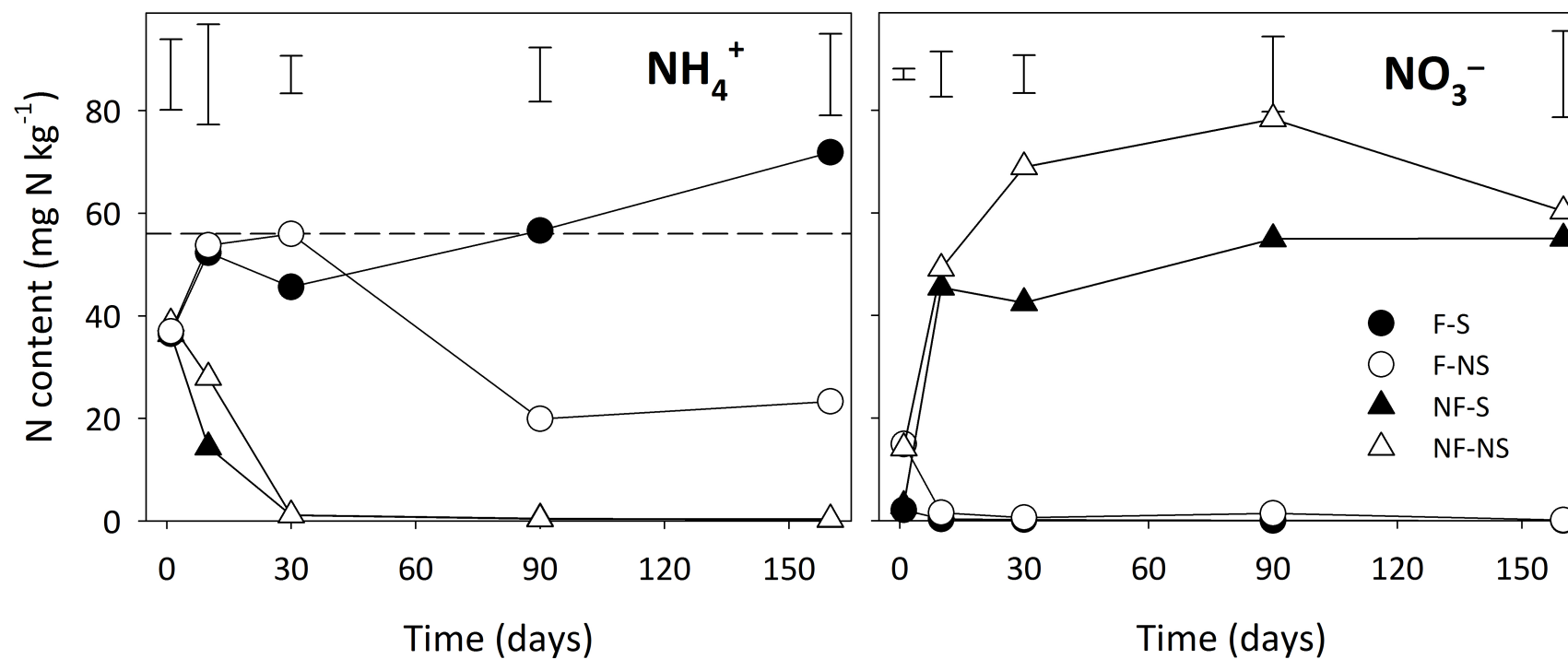


Figure 3: Variations in available inorganic N with time for soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition. Values for flooded samples represent the sum of floodwater and soil extractable N contents. Dashed line represents applied fertilizer-N. The error bars represent the least significant difference at $P = 0.05$ ($\text{LSD}_{0.05}$).

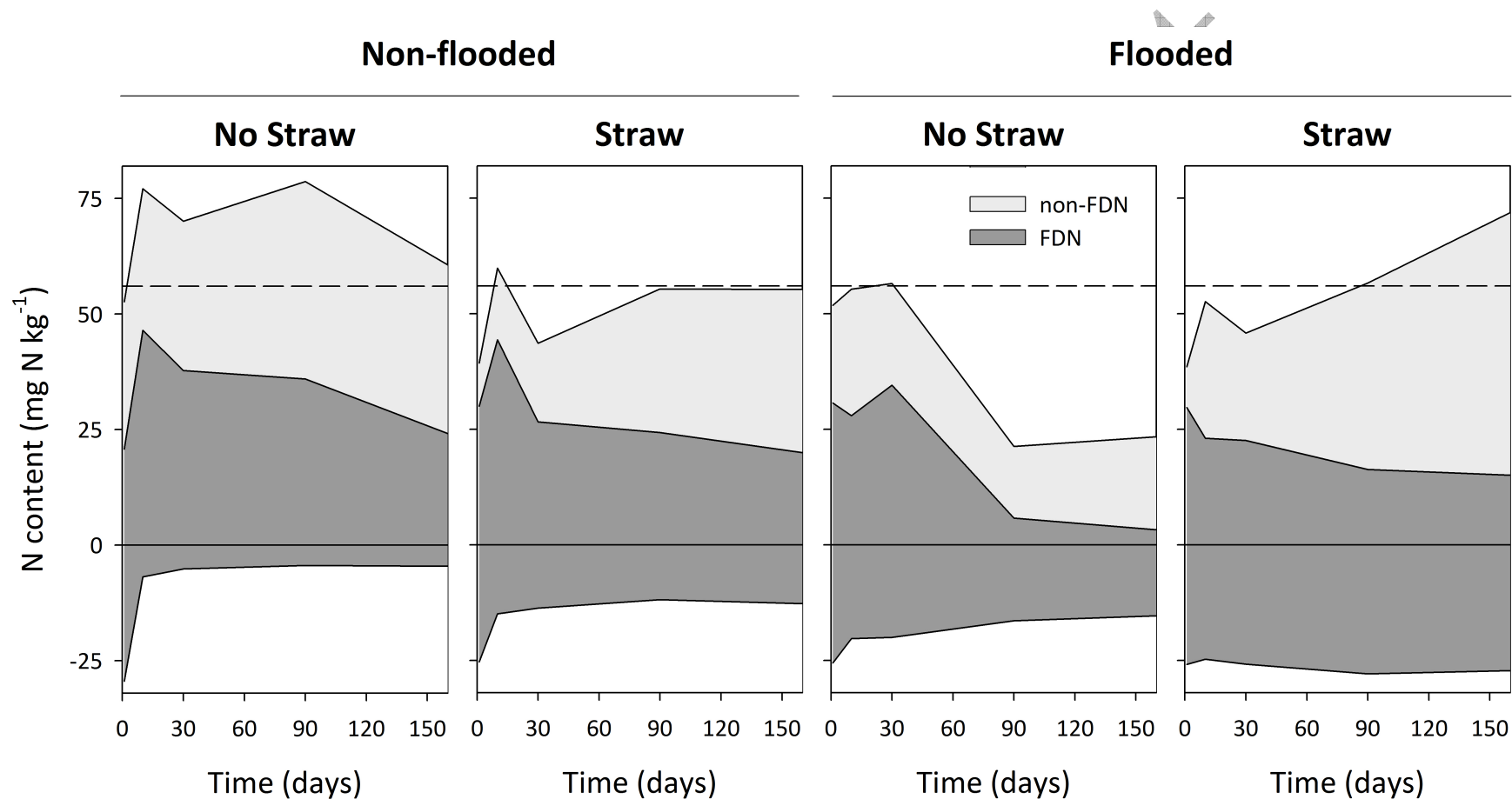


Figure 4: Variations in the (i) distribution of total available N (positive values) between fertilizer-derived (FDN), and soil or straw-derived (non-FDN) pools, as well as (ii) immobilized (negative values) fertilizer-derived N (FDN), with time in soils incubated under non-flooded or flooded conditions, without or with straw additions. Dashed line represents applied fertilizer-N.

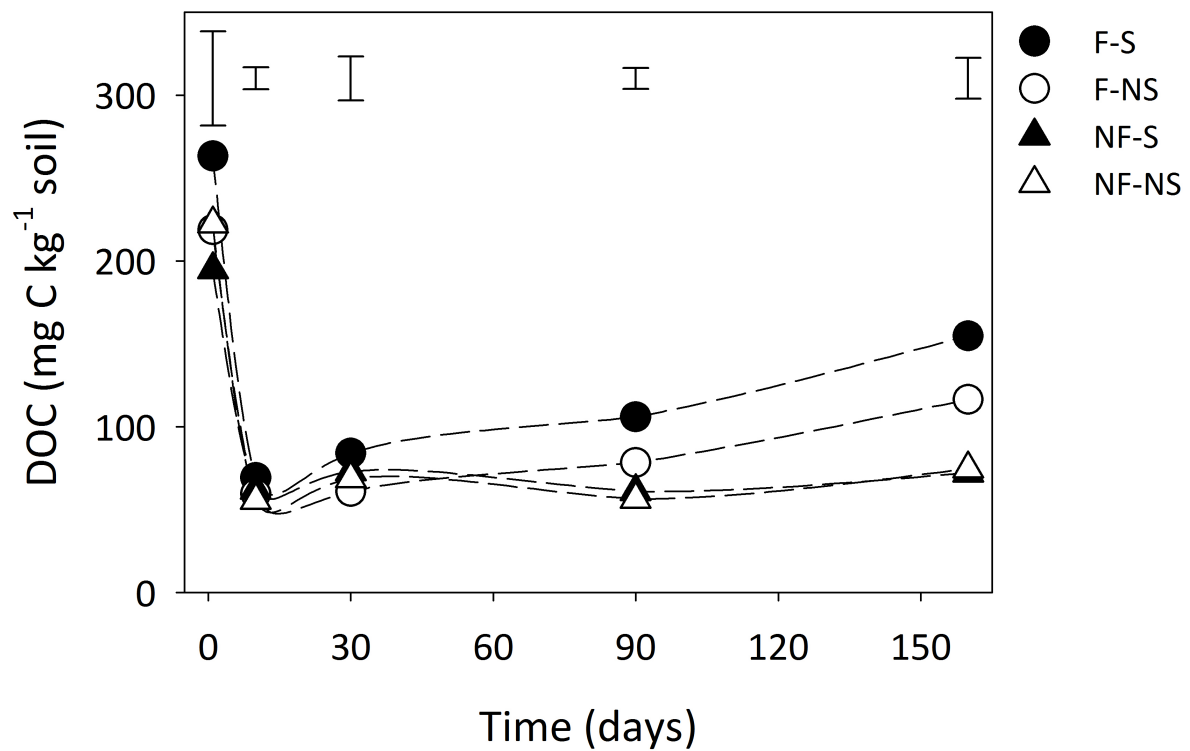


Figure 5: Variations in dissolved organic C contents with time for soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition. Values for flooded samples represent the sum of floodwater and soil water-extractable C contents. The error bars represent the least significant difference at $P = 0.05$ ($\text{LSD}_{0.05}$).