Nitrogen immobilization in paddy soils as affected by redox conditions and rice straw incorporation

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Article history:
Received 24 November 2012
Accepted 21 June 2013
Available online 18 July 2013

Keywords:
Fertilizer-derived nitrogen
Aggregate-size fractionation
Interlayer fixation
Amino sugars
Compound specific δ15N stable isotope mass spectrometry

Biotic and abiotic processes controlling nitrogen (N) immobilization in paddy soils may significantly affect nutrient availability for plant uptake during the rice cropping season, as well as the efficiency of applied N fertilizers. Understanding the influence of water and crop residue management practices on N availability, however, requires detailed insight into the mechanisms and factors controlling N immobilization in these soils. We evaluated changes in fertilizer-δ15N immobilization in a paddy topsoil incubated for 160 d under flooded or non-flooded conditions, with or without rice straw incorporation. The distribution of immobilized N between different soil fractions and interlayer N fixation was assessed by combining aggregate-size, density and chemical fractionation with stable isotope analysis, while compound-specific δ15N analysis of individual amino sugars was used to evaluate microbial utilization of applied N. Fast immobilization of applied N (∼48% applied N) was observed in both flooded (Eh = +0.4 to −0.2 V) and non-flooded (Eh = +0.4 to +0.6 V) soils, however in the latter most of this N was released during incubation. The finer soil fractions served as the greatest sink of immobilized N, retaining 5–36% of the added N. Although biotic processes were mainly responsible for N retention, about 4–11% of N applied to flooded soils was weakly fixed within the interlayer of clay minerals, primarily associated with microaggregates. Straw addition further enhanced N immobilization under both oxic and anoxic conditions, with ≈12% of total immobilized N (2–4% of applied N) associated with the light organic matter fraction. The increasing incorporation of applied N into microbial residues suggested that addition of rice straw to paddy soils may lead to effective microbial-mediated immobilization and stabilization of significant portions of N inputs.

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1. Introduction

Nitrogen (N) dynamics in paddy soils is perhaps one of the most complex biogeochemical cycles in agriculture, making N the most yield-limiting and difficult nutrient to manage in rice cropping systems worldwide (Eagle et al., 2000; Reddy, 1982). Although N supply drives productivity, poor N fertilizer-use efficiency (30–40% recovery of applied N) is characteristic of irrigated rice agro-ecosystems (Cassman et al., 2002). This has been largely attributed to rapid losses of applied fertilizer-N (Cassman et al., 1998; Ghosh and Bhat, 1998) and a greater degree of immobilization with respect to upland soils (Bird et al., 2001). Although research to reduce N losses through improved fertilizer management has received considerable attention in recent years (Cassman et al., 1998 and references within; Peng et al., 2010), detailed insight into the mechanisms and factors controlling N immobilization in flooded rice soils is still highly required to provide improved understanding of their impact on long-term soil fertility.

Biotic and abiotic processes controlling N immobilization may significantly influence N availability for rice crops as well as affect N losses. Inorganic N added to soil may be immobilized by the soil microbial biomass, primarily as amino and nucleic acids, and amino sugars (Nannipieri and Eldor, 2009). These microbial constituents may be subsequently remineralized or redistributed among more complex heterogeneous and less labile soil organic matter (SOM) fractions (Devêvre and Horwáth, 2001). Devêvre and Horwáth (2000) suggested that the larger and sustained microbial biomass generally found under anaerobic compared to aerobic conditions, may immobilize more N. Moreover, since microbial processes in arable soils are generally constrained by C availability, the incorporation of crop residues (CN = 50–70) into paddy soils between cropping seasons may possibly result in a further increase in N immobilization (Bird et al., 2001). Various studies have shown that crop residue incorporation into paddy soils may enhance microbial activity and size in both flooded and non-flooded conditions (Devêvre and Horwáth, 2000), and consequently lead to a significant increase in the amount of N immobilized. Therefore, understanding the factors influencing N immobilization in paddy soils is crucial for sustainable rice cropping systems.
immobilization of applied fertilizer-N into the biomass (Devère and Horwáth, 2001). Mineralization and immobilization of N by soil microorganisms are intimately coupled with the decomposition and stabilization of SOM, and it is the balance between these processes that defines the availability of N for plant uptake (Devère and Horwáth, 2001).

Abiotic processes may also contribute to N retention in soils, influencing N availability for plant uptake and feedback to microbial utilization. Relatively stable N-containing compounds may result from chemical reactions between ammonia and SOM (Knicker et al., 1997; Schmidt-Rohr et al., 2004). Olk et al. (2006) suggested that lignin-derived phenols which accumulate under prolonged anaerobic conditions may covalently bind nitrogenous compounds into recalcitrant forms, therefore contributing to the reduction in fertilizer and soil N availability in paddy soils. Ammonium fixation in layer silicates may also result in the immobilization of important amounts of recently applied N, particularly in poorly developed paddy soils that contain appreciable amounts of 2:1 clay minerals such as vermiculite and illite (Nieder et al., 2011; Wang et al., 2001).

Building upon these considerations, this work aims at providing insights into the immobilization of applied fertilizer-N as a function of soil redox conditions and rice straw incorporation. We hypothesize that (i) soil flooding may enhance N immobilization and influence the underlying mechanisms, and (ii) addition of labile OM with rice straw incorporation may influence microbially-mediated processes responsible for the immobilization of applied N.

To test these hypotheses we evaluated changes in fertilizer-15N immobilization in a paddy soil incubated for 160 d under flooded or non-flooded conditions, with or without rice straw incorporation. Certain soil fractions may have a large potential to selectively retain N by means of both biotic and abiotic processes. This may influence the distribution of immobilized N among fractions having different degrees of aggregation and mineralogical composition, as well as functionally distinct SOM pools with different characteristics and turnover rates (Christensen, 1992; Guggenberger et al., 1994). In order to provide important insights into the mechanisms controlling N immobilization in paddy soils, we quantified the partitioning of immobilized fertilizer N among different soil fractions, as well as interlayer N fixation, by combining aggregate-size, density and chemical fractionation procedures with stable isotope analysis. As plants do not synthesize appreciable amounts of amino sugars, these compounds are assumed to originate from the turnover of the soil microbial community, and therefore their quantification in soil is often adopted to indicate microbial contributions to SOM (Amelung, 2001; Parsons, 1981). These N containing biomarkers represent important constituents of the cell walls of bacteria, fungi and actinomycetes (Amelung, 2001), and as such may serve as a proxy for microbial N immobilization. Coupling amino sugar analysis to δ15N stable isotope mass spectrometry was therefore employed to evaluate the microbial utilization of applied N.

2. Materials and methods

2.1. Soil and plant materials

Soil material was collected from the Ap horizon (0–15 cm) of a paddy soil (Haplic Gleysol) within a long-term experimental platform in Vercelli, NW Italy (45°17′47″N, 8°25′51″E). The field has been under continuous single-cropped rice cultivation for the last 30 years, with crop residue incorporation in autumn, and field flooding for most of the cropping period (May–September). After removal of vegetation and bigger roots, the collected soil was passed through a 2-mm sieve without drying, and subsequently stored in cool, dark conditions until use. The main physical, chemical and mineralogical properties of the soil are reported in Table 1. Rice straw (Oryza sativa variety Sirio CL) was sampled after grain harvest from the same experimental platform, dried and cut into 1-cm segments. The total C and N contents of the straw were 400 g C kg−1 and 6.6 g N kg−1, respectively.

2.2. Microcosm design

The influence of soil redox conditions and rice straw incorporation on N dynamics was studied by means of a laboratory incubation experiment. The experimental design comprised a completely randomized 2 × 2 factorial arrangement representing (i) soil incubated under non-flooded (NF; moisture content kept at 50% water-holding capacity; Eh values between +0.4 and +0.6 V) or flooded (F; submerged under 5 cm of degassed and deionized water; Eh values from +0.4 to −0.2 V) conditions, (ii) without (NS) or with (S) the addition of rice straw (application dose of 4.3 g kg−1 soil equivalent to 10 Mg ha−1 d.w.). After a 7 d pre-incubation period, inorganic N was added to the soils as enriched (NH4)2SO4 (10 atom% 15N). An application dose of 56 g N kg−1 soil was used, equivalent to 130 kg N ha−1 generally applied as mineral fertilizer in the field. A second set of soil samples was treated with natural abundance (NH4)2SO4 to account for isotope fractionation. Incubation was carried out in the dark for 160 d at 25 °C, and triplicate soil samples destructively sampled after 1, 10, 30, 90 and 160 d from the application of fertilizer.

2.3. Chemical analyses and physical soil fractionation

Inorganic N concentrations in floodwater and 0.5 M K2SO4 soil extracts were determined spectrophotometrically, while the amount of immobilized fertilizer-N was calculated from the δ15N values of soil samples after removal of K2SO4 extractable N. The distribution of immobilized fertilizer-N among soil fractions was assessed by a combination of aggregate-size and density fractionation on samples collected on 1, 10 and 160 d of incubation. This was done using a method adapted from Six et al. (2000, 2001). Briefly, 60 g of soil was submerged in deionized water on a 250 μm sieve for 15 min. Slae-resistant micro- and macroaggregates were separated by moving the sieves up and down 3 cm every 2 s for 2 min. The microaggregates (<250 μm) were subsequently dispersed by suspending in 0.1% Na-hexametaphosphate and treating ultrasonically with 440 J ml−1 using a probe-type sonicator. Fine sand and coarse silt (250–20 μm), and clay and fine silt (<20 μm) fractions were subsequently isolated by sedimentation. An aliquot (±25 g) of the macroaggregates (<250 μm) was suspended in 1.6 g cm−3 Na-polytungstate (1:5 soil:solution ratio) to isolate the light organic matter (OM) fraction. After flotation and removal of the light fraction, the heavy part was rinsed in deionized H2O, dispersed in 0.1% Na-hexametaphosphate and treated ultrasonically with 440 J ml−1. The coarse sand-sized fraction (2000–250 μm) present

### Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC (g kg−1)</td>
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</tr>
<tr>
<td>TN (g kg−1)</td>
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</tr>
<tr>
<td>NO3 (mg N kg−1)</td>
<td>5.5</td>
</tr>
<tr>
<td>NH4 (mg N kg−1)</td>
<td>3.3</td>
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<td>pH</td>
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<td>CEC (cmol kg−1)</td>
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<td>K+ exchangeable (meq kg−1)</td>
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<tr>
<td>Fe0−8 (g kg−1)</td>
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</tr>
<tr>
<td>Fe3+ (g kg−1)</td>
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<tr>
<td>Al3+ (g kg−1)</td>
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</tr>
<tr>
<td>Sand (g kg−1)</td>
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</tr>
<tr>
<td>Silt (g kg−1)</td>
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<tr>
<td>Clay (g kg−1)</td>
<td>65.0</td>
</tr>
<tr>
<td>WHC (g kg−1)</td>
<td>405</td>
</tr>
</tbody>
</table>

a Organic carbon.
b Total nitrogen.
c Cation exchange capacity determined by BaCl2 (pH 8.1).
d Dithionite-citrate-bicarbonate minus oxalate extractable Fe.
e NH4-oxide-extractable Fe and Al.
f WHC: water-holding capacity determined gravimetrically.
g Determined by XRD analysis.

[Table 1](#)
in the macroaggregate was subsequently separated by sieving, while the fine sand and coarse silt (250–20 μm), and clay and fine silt (<20 μm) fractions were isolated by sedimentation as for the microaggregates. A total of six fractions were thus obtained including: (i) light fraction (LF); (ii) coarse sand-sized fraction (M2000-250); (iii) fine sand and coarse silt-sized fraction of macroaggregates (M250-20); (iv) fine silt and clay-sized fraction of macroaggregates (M < 20); (v) fine sand and coarse silt-sized fraction of microaggregates (m250-20); and (vi) fine silt and clay-sized fraction of microaggregates (m < 20). All fractions were washed thoroughly with deionized H2O (electrical conductivity <50 μS cm−1), dried at 40 °C, their yield measured and finally ground for analysis.

Total C and N contents and stable N isotope composition of K2SO4 treated soils and all soil fractions were determined with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (FlashEA-1112 equipped with a thermal conductivity detector and interfaced via a ConFlo III to a DeltaPlus XP system, Thermo Electron Corporation, Bremen, Germany). Since a carbonate-free soil was evaluated by HF:HCl treatment followed by microdiffusion of the re-dissolved supernatant in methanol. After addition of 100 μl of 2 M KOH and an acid trap prepared with 5% dimethylchlorosilane in toluene for at least one week, a constant discharge into the IRMS for 20 s via the open split, as a reference gas for the calculation of relative isotope ratios of individual compounds, and this setup was previously shown to be most appropriate for correcting injection–dependent isotope fractionation (Schmidt et al., 2003). Chromatographic separation of aldononitrile acetate derivatives was performed on a 60 m × 0.25 mm i.d. fused silica capillary column coated with 5% diphenyl- and 95% dimethyl-polysiloxane stationary phase (Supelco SPB-5, 0.25 μm film thickness). The oven temperature was held at 120 °C for 1 min, increased to 250 °C at 10 °C min−1, held at 250 °C for 2.5 min, increased to 270 °C at 20 °C min−1, held at 270 °C for 2 min and finally ramped to 300 °C at 50 °C min−1 and kept there for 5 min. Quantification and recovery determination were carried out against the internal standards myo-inositol and N-methylglucamine, respectively.

2.5.2. Compound-specific δ15N analysis

Compound-specific δ15N analysis of individual amino sugars was performed using a gas chromatograph–combustion–isotope ratio mass spectrometer (GC–C–IRMS) system consisting of a Thermo GC Ultra interfaced via a GC Combustion Interface III to a Delta V Advantage continuous flow IRMS (Thermo Electron Corporation). The GC was equipped with a split/splitless injector kept at 250 °C and with a gooseneck liner for splitless injection (length 10.5 cm, 5 mm i.d. for 9.2 cm, without glass wool packing), which was previously deactivated with 5% dimethylchlorosilane in toluene for at least one week. A constant sample volume of 2 μl (~2–8 nmol of each amino sugar) was injected splitless for 1 min, with an autosampler (TriPlus, Thermo Finnigan MAT, Bremen, Germany) equipped with a 50-mm needle length. Injector liner, needle length and splitless time are known to influence isotope ratios of individual compounds, and this setup was previously shown to be most appropriate for correcting injection–dependent isotope fractionation (Schmitt et al., 2003). Chromatographic separation of aldononitrile acetate derivatives was performed on a 60 m × 0.25 mm i.d. fused silica capillary column coated with 5% diphenyl- and 95% dimethyl-polysiloxane stationary phase (Agilent DB-5 ms, 0.25 μm film thickness) adopting a temperature programme starting at 120 °C for 1 min, increased to 230 °C at 20 °C min−1, held at 230 °C for 22 min, increased to 260 °C at 20 °C min−1, held at 260 °C for 8 min and finally ramped to 300 °C at 60 °C min−1 and kept there for 5 min. The connection to the Combustion Interface III (Thermo Finnigan MAT) was through a borosilicate press-fit Y-splitter that, together with a backflush valve supplying He, allowed for the removal of solvent peaks from the effluent of the GC column. When the backflush valve is closed the column effluent passes through an oxidation furnace composed of braided nichrome/copper oxide wire kept at 950 °C and a reduction furnace of braided copper wire kept at 650 °C, followed by a H2O/CO2 trap at liquid N2 temperature before reaching the IRMS.

During all measurements, six pulses of N2 gas were directly discharged into the IRMS for 20 s via the open split, as a reference gas for the calculation of relative δ15N values and drift correction during measurements. δ15N values of individual amino sugar derivatives, calculated from five replicate measurements were calibrated against internal standard N-methylglucamine and corrected for the N added during derivatization and amount dependence using the δ15N values of standard amino sugars determined by EA–IRMS as described in detail by Glaser and Amelung (2002).

2.6. Spectroscopic characterization of light fractions

Solid state 15N nuclear magnetic resonance (NMR) spectroscopy with cross polarization/magic angle spinning (CPMAS) was adopted
to investigate the composition of fertilizer-derived $^{15}$N in the light fractions. NMR spectra were acquired on a Bruker Avance II 400 MHz spectrometer with a frequency of 40.55 MHz for $^{1}$H and 400.23 MHz for $^{1}$H, while CP/MAS acquisition parameters as described by DiCosty et al. (2003) were adopted. These involved a 90° pulse to $^{1}$H applied for 3.05 μs, a contact time of 800 μs, 10 ms acquisition time with a decoupling frequency of 75 kHz, 1 s delay time and 100 Hz line-broadening. LF samples (85 mg) were spiked with 40 mg of $\text{(NH}_4\text{)}_2\text{SO}_4$ as an internal standard, packed into a 4 mm diameter rotor and spun at a frequency of 9 kHz. About 244,000 scans were acquired for each sample.

3. Results and discussion

3.1. Available and immobilized N

Soil redox conditions and the application of rice straw influenced N availability in soils (Table 2). Incubation of soils under non-flooded conditions in the absence of straw resulted in a rapid decrease in available N with respect to initial contents (64.9 ± 0.9 mg N kg$^{-1}$). However, after only 10 d, available N was equal to or greater than initial contents. These results suggest that after an initial fast net immobilization, the degradation of SOM eventually led to a net N mineralization. The addition of rice straw (C/N = 61) resulted in lower available N concentrations with respect to the initial inorganic N content throughout the incubation. In fact, available N concentrations in samples receiving straw were always lower than those in samples without straw suggesting a greater net N immobilization in the presence of labile OM. Under flooded soil conditions contrasting trends in available N contents were observed. Flooded soils without straw showed unchanged contents of available N over the first 30 d of incubation, followed by a drastic decrease thereafter. N availability in these soils was lower than in non-flooded soils without straw. This is in contrast with the widely held notion that net ammonification is generally greater under anaerobic than aerobic conditions because of the low metabolic N requirement of anaerobic bacteria (Reddy and deLaune, 2008). This suggests that N immobilization could nevertheless play an important role under anaerobic conditions, although loss of fertilizer-N through nitrification-denitrification reactions could not be excluded (Devêvre and Horwáth, 2001). In their study on N mineralization and immobilization in different soils, Wang et al. (2001) reported that net N mineralization was not always higher under waterlogged with respect to aerobic conditions since it depends on the balance of processes including mineralization, immobilization, clay fixation, and losses by nitrification–denitrification and volatilization. The addition of straw to flooded soils enhanced N availability with incubation time, suggesting that the rate of release of inorganic N during the mineralization of straw-derived OM was faster than the rate of immobilization and loss.

$^{15}$N values of K$_2$SO$_4$ treated soils allowed for the calculation of the amount of immobilized FDN (Table 2). In all cases, approximately 48% of the applied N was immediately immobilized at the beginning of the incubation irrespective of redox conditions or addition of rice straw. Many studies have documented a rapid immobilization of applied NH$_4^+$ into non-exchangeable pools via abiotic processes and microbial immobilization 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. Except for flooded soils receiving straw, the amount of immobilized fertilizer-N tended to decrease with incubation time, and after 160 d of incubation immobilized N followed the order NF-NS < NF-S < F-NS < F-S with 8, 23, 27 and 49% of the applied N being immobilized, respectively. These results suggest that both lower soil redox conditions and the addition of labile OM may enhance the immobilization of applied fertilizer-N in paddy soils.

3.2. Distribution of immobilized N among different soil fractions

Physical fractionation of soil samples allowed for the evaluation of the distribution of immobilized N between different soil fractions, thus providing an insight into the importance of different N immobilization mechanisms involved. Grouped results obtained from the analyses of all soil samples show that the C/N ratio of the different fractions was largest in the LF and decreased with both aggregate- and particle-size in all treatments (Fig. 1). Larger C/N ratios were obtained for the ~20 and 250–20 μm size fractions in macroaggregates (M < 20 and M250–20) with respect to the corresponding fractions in microaggregates (m < 20 and m250–20). This suggests that macroaggregates contained more recent, partially stabilized OM, while OM associated with microaggregates was enriched in microbial products and showed a higher degree of decomposition. The application of rice straw to flooded and non-flooded soils contributed recent material to the LF resulting in an increase in the mean C/N ratio with respect to soils without straw. Moreover, in soils that received straw both the C content and C/N ratio of the LF decreased with incubation time (from a mean C content of 1.1 to 0.6 mg C g$^{-1}$ and a C/N of 26 to 18 on days 1 and 160, respectively) reaching final values similar to those obtained for soils without straw (0.5 mg C g$^{-1}$ and C/N = 17). This confirmed the rapid turnover of this fraction under both aerobic and anaerobic conditions. On the other hand, C contents and C/N ratios of the other fractions were neither influenced by straw addition nor flooding, and did not show any variations with incubation time.

Although across all treatments the LF had the largest C (110.5–253.5 mg C g$^{-1}$ fraction) and N (6.7–25.3 mg N g$^{-1}$ fraction) contents, this fraction represented only 3.5–11.9% and 2.0–4.8% of total soil C and N, respectively. On the other hand, the finer soil fractions (M < 20 and m < 20) together represented 68–79% and 78–85% of the total C and N, respectively. Non-flooded soils had more C associated with the fine soil fractions present within the slake-resistant macroaggregates (M < 20) with respect to microaggregates (m < 20), while the opposite was true for flooded soils. In fact, in the latter mean yields as well as C and N contents of m < 20 size fractions were generally greater than those obtained for M < 20 size fractions, both in the presence or absence of straw (Fig. 1). These results suggest that the predominant mechanism of SOM stabilization may be ascribed to the close associations between OM and mineral phase in the finer soil fractions. Moreover, macroaggregate stability may be somewhat compromised in flooded soils possibly due to the disruptive energy occurring upon slaking (Six et al., 2000; Zanini et al., 1998), reductive dissolution of Fe oxides (Kögel-Knabner et al., 2010), reduced growth of fungal mycelia under anaerobic conditions (Polyanskaya et al., 2010), or soil compaction during the preparation of the trials. Recovery of immobilized FDN, calculated as the ratio of the sum of fertilizer-N immobilized in the different fractions (Fig. 2) to the total

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation time (d)</th>
<th>LSD$_{0.05}$^c</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Non-flooded, without straw (NF-NS)</td>
<td>Total available N$^a$</td>
<td>52.7 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>Immobilized FDN$^b$</td>
<td>29.4 ± 6.9</td>
</tr>
<tr>
<td>Non-flooded, with straw (NF-S)</td>
<td>Total available N</td>
<td>39.3 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>Immobilized FDN</td>
<td>25.3 ± 4.9</td>
</tr>
<tr>
<td>Flooded, without straw (F-NS)</td>
<td>Total available N</td>
<td>51.8 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>Immobilized FDN</td>
<td>25.6 ± 2.0</td>
</tr>
<tr>
<td>Flooded, with straw (F-S)</td>
<td>Total available N</td>
<td>38.5 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>Immobilized FDN</td>
<td>25.9 ± 2.4</td>
</tr>
</tbody>
</table>

$^a$ Determined as the sum of NH$_4^+$ and NO$_3^-$ in soil extracts and floodwater (in flooded soils).

$^b$ Fertilizer derived N determined after removal of K$_2$SO$_4$ extractable N.

$^c$ Least significant difference for P < 0.05.
immobilized N (Table 2), ranged from 71 to 90% suggesting that the amount of immobilized N lost during the fractionation procedure was limited. Only 1–4% of applied fertilizer-N (8–16% of total immobilized FDN) was associated with the coarser mineral fractions (M2000-250, M250-20 and m250-20) with no differences between treatments. In contrast, the finer soil fractions in both macro- and microaggregates (M < 20 and m < 20) served as the greatest sink of applied N in all treatments, together retaining 62–91% of the total immobilized FDN. Similar findings were reported by Hagedorn et al. (2005) who showed that about 50–60% of the immobilized 15N applied to forest soils occurred in the finer soil fractions with small C/N ratios. Rapid transfer of added N onto such soil fractions is often attributed to microbial immobilization (Hagedorn et al., 2005), since most of the soil microbial biomass is associated with small-sized separates (Kandeler et al., 2000; Kögel-Knabner et al., 2008) that are also dominated by products of microbial resynthesis (Guggenberger et al., 1994). However, considering that the soil used in this experiment contained a vermiculite- and illite-dominated clay fraction (Table 1), fast, diffusion controlled, abiotic fixation of NH4+ may be also responsible for significant immobilization of applied N on finer soil fractions, particularly under flooded soil conditions (Nieder et al., 2011). Trends in FDN immobilized on the finer soil fractions during incubation for each treatment were similar to those observed for total immobilized FDN. With time, the sum of FDN in M < 20 and m < 20 fractions decreased more rapidly in non-flooded than flooded soils, while straw addition generally enhanced FDN retention in these fractions, especially under flooded conditions.

Moreover, results evidenced that in non-flooded soils more immobilized FDN was associated with the fine soil fractions present within macroaggregates with respect to microaggregates, while in flooded soils the opposite was true. Aggregate ratios were generally <1 in non-flooded and >1 in flooded soils (Table 3) suggesting that the lower degree of aggregation in the latter could be responsible for the greater amount of applied N immobilized on microaggregates. However, the different 15N isotope ratios and fDN values obtained for the m < 20 and M > 20 fractions also suggest that aggregation may nonetheless influence N immobilization processes. In fact, in flooded soils enrichment ratios evidenced a preferential immobilization of applied N on microaggregates (values > 1; Table 3). With time these ratios tended to decrease both with and without straw incorporation, reaching values close to unity in the former. In contrast, in non-flooded soils N immobilization on microaggregates was favoured at the beginning of the incubation but strongly shifted towards a preference for macroaggregates after 10 d of incubation (values < 1; Table 3). These results seem to suggest that preferential and rapid immobilization of FDN on the finer soil fraction within microaggregates could be due to their greater surface area or lower diffusive inhibition with respect to macroaggregates. These properties could strongly influence abiotic N immobilization mechanisms. Scherer and Zhang (1999) previously reported that coating of the surface of clay minerals by Fe oxides may limit the diffusion of NH4+ into or out of the interlayer of clay minerals. Moreover, the major processes determining aggregate stability under flooded soil conditions often involve iron bridging and formation of iron cutans (Kögel-Knabner et al., 2010). Thus, it is plausible to hypothesize that aggregation could significantly limit interlayer NH4+ fixation and release when 2:1 clays are present. On the other hand, since water-stable macroaggregates are generally considered to be “hotspots” of microbial activity (Bronick and Lal, 2005; Jiang et al., 2011), the decreasing trend in enrichment ratios with incubation time (Table 3) could have also possibly resulted from an enhanced biotic immobilization of N in macroaggregates, particularly under oxic soil conditions. However, the expected influence of substrate addition in samples receiving straw was not observed.
Variations in aggregate yield and enrichment ratios for the fine silt and clay-sized fraction (<20 μm) in micro- to macroaggregates with time in soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield ratio</th>
<th>Enrichment ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 d</td>
<td>10 d</td>
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<tr>
<td>Non-flooded, without straw (NF-NS)</td>
<td>0.64</td>
<td>0.60</td>
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<tr>
<td>Non-flooded, with straw (NF-S)</td>
<td>0.49</td>
<td>0.67</td>
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<tr>
<td>Flooded, without straw (F-NS)</td>
<td>0.85</td>
<td>1.56</td>
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<tr>
<td>Flooded, with straw (F-S)</td>
<td>1.66</td>
<td>1.66</td>
</tr>
</tbody>
</table>

* Calculated as the ratio of mass yields (in g fraction kg⁻¹ soil) of m < 20 to M < 20 fractions.
* Calculated as the ratio of \( f_{\text{from}} \) of m < 20 to M < 20 fractions.

Nevertheless, in both non-flooded and flooded treatments, straw addition resulted in enhanced FDN immobilization on the light fraction (≈12% of total immobilized FDN) with respect to soils not receiving straw (Fig. 2). Although the LF was rapidly decomposed during incubation under both oxic and anoxic soil conditions (as described above), FDN associated with this fraction tended to increase with time. This suggests that applied N was actually immobilized during degradation of the LF, although it was not possible to distinguish between microbiologically mediated uptake of applied N or abiotic reactions between added N and lignin-derived phenols present in the decomposing rice straw. The smaller values of \( f_{\text{from}} \) observed for the LF in flooded with respect to non-flooded samples nonetheless agree well with the widely held notion that less mineral-N is assimilated into microbial biomass during the anaerobic decomposition of organic substrate in contrast to aerobic conditions which are generally characterized by higher microbial N requirements (Wang et al., 2001). The significant role of the LF pool in cycling recently added N was also reported by Bird et al. (2002) who showed rapid enrichment of this fraction in straw incorporated plots after application of \(^{15}\)N fertilizer. Similarly, findings from the short-term incubations with forest soils presented by Compton and Boone (2002) suggest that a large portion of the rapid NH\(_4\)\(^{+}\) sink (35–55% of added NH\(_4\)\(^{+}\)) involves the LF. In both cases, however, the authors were not able to discern between microbial and abiotic mechanisms although they hypothesized that, since the specific surface area of the relatively undecomposed LF organic matter is expected to be small, sorption reactions may be less important than microbial activity as a mechanism for N incorporation into this fraction.

### 3.3. Abiotic N immobilization on clay minerals

The soil used in this work had a mean fixed NH\(_4\)\(^{+}\) content of 51 mg N kg\(^{-1}\), approximately 4.6% of the total N, but over 15 times the amount of exchangeable NH\(_4\)\(^{+}\) before application of fertilizer-N (Table 1). Moreover, the presence of vermiculite and illite (Table 1), as well as the low percentage K\(^{+}\) saturation (≈2.8%), suggest that interlayer NH\(_4\)\(^{+}\) fixation may strongly influence N availability in this soil (Wang et al., 2001). The amount of FDN fixed within the clay minerals present in the finer soil fractions (Fig. 3) evidences that clay fixation was responsible for the rapid immobilization of 10–13% of applied N in all treatments. This explained about 34% of the total immobilized FDN after only 1 d of incubation, irrespective of redox conditions or addition of rice straw. In fact, NH\(_4\)\(^{+}\) fixation in vermiculite is known to be a fast reaction occurring at a similar rate to the NH\(_4\)\(^{+}\) exchange reaction on surface sites (Shen et al., 1997). Under aerobic soil conditions most of the fixed fertilizer-derived NH\(_4\)\(^{+}\) was rapidly released within 10 d of incubation both in the presence and absence of straw, reaching values as low as 0.9 mg N kg\(^{-1}\), barely 1% of the applied N. These results suggest that under oxic conditions nitrification may promote the release of NH\(_4\)\(^{+}\) from the clay mineral interlayers (Green et al., 1994). This lends support to the notion that recently fixed NH\(_4\)\(^{+}\) may nonetheless be weakly retained along the edges of clay mineral interlayers, and thus prone to release and subsequent utilization by plants or microorganisms (Nieder et al., 2011 and references therein).

In contrast, in flooded soils fixed fertilizer-N was released to a lesser extent with respect to non-flooded soils. After 160 d of incubation, fixed FDN explained approximately 16 and 20% of total immobilized FDN equivalent to about 7 and 4% of applied fertilizer-N in flooded soils with and without straw addition, respectively. Similar results were obtained by Devêvre and Horwáth (2001) who estimated that 1 to 5% of fertilizer-derived N was immobilized by abiotic clay fixation. Since the release of fixed NH\(_4\)\(^{+}\) is generally assumed to be diffusion controlled, the inhibition of nitrification under anaerobic conditions, responsible for greater exchangeable NH\(_4\)\(^{+}\) concentrations, probably resulted in a slower release of fixed FDN with respect to oxic soils. However, we cannot rule out that this process was underestimated with respect to field conditions where plant uptake would further contribute to reducing available NH\(_4\)\(^{+}\) concentrations. Moreover, NH\(_4\)\(^{+}\) fixation in flooded paddy soils was also found to be closely related to the declining redox potential (Schneiders and Scherer, 1998). The decreasing Eh (from +350 to +10 mV) and increasing pH (from 5.9 to 7.1) measured during the first 90 d of incubation under flooded conditions could have brought about the reductive dissolution of Fe (hydr)oxides coated on the surface of clay minerals, favouring the diffusion of NH\(_4\)\(^{+}\) ions into the interlayers (Nieder et al., 2011).

The addition of straw to flooded soils resulted in significant increase in available N with incubation time (Table 2), probably due to the mineralization of the added labile OM. This increase in exchangeable NH\(_4\)\(^{+}\) could have further limited the diffusion of NH\(_4\)\(^{+}\) out of the clay mineral interlayers, thus explaining the higher amounts of fixed FDN in these soils. Moreover, the low Eh values measured during incubation (as low as −180 mV after only 30 d with pH values around 7) suggest that the supply of readily oxidizable OM (i.e. electron donors) with the incorporation of crop residues could have further stimulated the microbially-catalyzed reductive dissolution of Fe (hydr)oxides (Katoh et al.,

![Fig. 3. Variations in the total contents of interlayer fixed fertilizer-derived N and its distribution between the fine fractions of macro- (M = 20) and microaggregates (m = 20) with time in soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition.](image-url)
Although amino sugars may constitute an important part of the newly immobilized N (He et al., 2006) and may adequately represent the degree of N sequestered within soil microbial residues (Amelung, 2001; Decock et al., 2009), recent investigations have shown that a significant portion may be present as microbial residues stabilized in soil and not part of living microbial cells (Amelung, 2001; Glaser et al., 2004; Liang et al., 2007). Since the turnover time of these cell wall constituents in soil is suggested to be much longer than that of living microorganisms (Glaser et al., 2006), variations in their total concentrations may not necessarily reflect actual microbial activity. The application of compound-specific δ15N isotope analysis of amino sugars, therefore allowed not only for differentiation between old and new bacterial and fungal residues, but also to trace and quantify the incorporation of enriched fertilizer-N into the active microbial biomass. Incorporation of applied N into the amino sugar-N pool (Fig. 4) was calculated from the 15N isotopic enrichment of glucosamine and galactosamine, since mannosamine and muramic acid were generally below the limits of quantification for GC-C-IRMS analysis.

Under non-flooded conditions, isotopic enrichment of the amino sugars increased with incubation time, particularly in soils receiving straw (Fig. 4). Incorporation of FDN into the amino sugar pool was from 6.5 to 10 times greater in soils receiving straw with respect to those not receiving straw, even though fertilizer-derived amino sugar-N accounted for only a minor portion (0.6–1.3%) of the total amino sugar-N pool. After 30 d of incubation about 0.6 mg FDN kg⁻¹ was incorporated into the amino sugars in non-flooded soils with straw. This accounted for about 9% of the increase in total amino sugar-N calculated for this treatment over the same period of time, assuming that this increase was totally due to microbial growth. These results suggest that under aerobic soil conditions, application of rice straw stimulated microbial growth due to the presence of labile substrate, that in turn resulted in significant biotic N immobilization. However, only a small portion of the N utilized for microbial growth actually accounted for the applied N, with the majority of the incorporated N probably originating from the degrading straw.

Under flooded soil conditions, variations in total amino sugar contents showed different trends with respect to non-flooded conditions (Fig. 4). In fact, in both flooded treatments without or with straw, amino sugar contents tended to decrease with time resulting in a loss of ~9 mg N kg⁻¹ over the incubation period. This loss was more rapid in the soil samples with straw compared to those without straw, particularly in the first 90 d of incubation. This is in contrast with the findings of Devêvre and Horwáth (2001) that report that the incorporation of a significant amount of C substrate in the form of rice straw increased microbial activity and size dramatically, and led to an increase in biomass-N under flooded soil conditions. Moreover, the depletion in amino sugar-N proportions generally attributed to conditions of substrate limitations (Amelung et al., 1999; Roth et al., 2011) was not expected to be responsible for the observed trend, particularly in the soils that received both straw and inorganic N. Therefore, we suggest that the decrease in total amino sugars under flooded soil conditions could be attributed to a net loss possibly due to substantial desorption of previously stabilized microbial residues and subsequent mineralization or loss to floodwaters. Previous studies have suggested that free amino sugars may only weakly sorb to the soil solid phase (Roberts et al., 2007), whereas the strong sorption and stabilization of these N-containing compounds onto clay-sized mineral fractions (Glaser et al., 2006; Kandelers et al., 2000; Zhang et al., 1998) is mainly attributable to the presence of acidic functional groups present on the peptidoglycan microbial residues (Kaiser and Zech, 2000). This binding mechanism would probably explain the rapid desorption of these microbial residues with increasing pH and decreasing Eh observed during the incubation of flooded soils receiving straw, and to a lesser extent those not receiving straw. We further speculate that the release of microbial residues-N under anoxic conditions and consequent loss of organic N

Fig. 4. Variations in total (sum of glucosamine, galactosamine, mannosamine and muramic acid) and fertilizer-derived (sum of glucosamine and galactosamine) amino sugar N with time in soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition. Error bars represent the standard error.
Total amino sugar-N contents decreased under anoxic conditions, their isotopic enrichment tended to increase during incubation (Fig. 4), confirming that applied fertilizer-N was nonetheless incorporated into these N-containing microbial constituents. Amino sugar FDN was from 2 to 2.7 times greater in soils receiving straw with respect to those not receiving straw, although the incorporation of applied N was limited with respect to non-flooded soils, particularly in the first 90 d of incubation. However, after 90 d both treatments with or without straw showed a significant increase in amino sugar FDN values. This could have been due to a shift in the microbial community structure leading to the establishment of a biomass with higher N requirements. Various authors report that upon flooding, changing environmental conditions resulting from the decreasing availability of electron acceptors and simple C sources for growth, could lead to a shift from copiotrophic to oligotrophic bacterial populations (Bai et al., 2000; Noll et al., 2005). Similarly, the proliferation of gram-positive bacteria with thicker cell walls as the major decomposers of rice straw in non-paddy soils, observed by Roth et al. (2011).

Due to their specific occurrence in cell wall structures of fungi and bacteria, the GluN:GalN ratio has been frequently used to cautiously estimate the relative contributions of these microbial groups to SOM turnover and accumulation (Amelung, 2001; Liang et al., 2007; Zhang and Amelung, 1996). A mean value of 1.9 was obtained for the total amino sugar GluN:GalN ratio, with no differences between treatments (Fig. 5). Moreover, ratios failed to show any temporal variations in any of the treatments investigated. Considering that cultivated bacteria and fungi were reported to have GluN:GalN ratios of about 1.9 and 4.6, respectively (Glasser et al., 2004), the relatively low measured ratios were probably indicative of a dominance of bacterial over fungal microbial residues in the paddy soil used for the experiment. This agrees with the well known fact that anoxic soil conditions established during the flooding of rice paddies tend to inhibit fungal proliferation (Bossio and Scow, 1998; Reddy and deLaune, 2008). However, the higher GluN:GalN ratios for fertilizer-derived amino sugar-N observed for non-flooded soils receiving straw (Fig. 5), suggested that the fungal biomass present in these soils could have been partly responsible for the biotic immobilization of applied N under oxic conditions, although this had no effect on total GluN:GalN ratios. On the other hand, addition of straw to flooded soils did not have a clear effect on fertilizer-derived GluN:GalN ratios, although it resulted in a greater variability with values ranging from 1.6 to 2.8. This increased variability might well result from changes in the composition of the bacterial community since even gram-positive and gram-negative bacteria may have different amino sugar contents (Amelung, 2001).

Biotic immobilization of applied N and distribution among different soil fractions was evaluated by correlating amino sugar FDN to soil FDN. Among all the soil fractions analysed only fertilizer-N immobilized on the LF was significantly correlated to the amino sugar FDN (r = 0.896, P < 0.001; Fig. 6). It could be therefore concluded that the transfer of applied N onto the LF was strictly related to biotic immobilization mechanisms as a result of microbial growth during the decomposition of labile substrates. Although the maximum amino sugar contents generally occur in the clay fractions (Glaser et al., 2006; Guggenberger et al., 1999), these results suggest that the labile OM pool serves as an important “hotspot” for microbial N transformations and plays a significant role in determining N availability. The degree of decomposition and disintegration of incorporated rice straw was previously reported to be correlated to the microbial colonization of the leaf sheaths (Kimura and Tun, 1999). Moreover, scanning electron microscopy and transmission electron microscopy of soil sections have evidenced that bacteria are often largely confined to the periphery of degrading plant residues (Nannipieri and Eldor, 2009). The biotic immobilization of applied N onto decomposing plant residues was also confirmed by 15N CPMAS NMR spectra of natural abundance rice straw and the 15N-enriched light fractions separated from non-flooded and flooded soils receiving straw (Fig. 7). In fact, during incubation under both redox conditions, spectra evidenced an increase in intensity of the signal between 73 and 108 ppm with maximum intensity at 96 ppm attributable to amide N of proteins (Knicker et al., 1997). The predominance of NMR signals from products of biological origin, particularly N in peptide linkages, suggests that microbial activity on the LF could have been responsible for the immobilization of applied N. These results are in line with the findings of Knicker et al. (1997) that report the uptake of applied NH4+ and synthesis of microbial proteins and biological N-containing structures as the major pathway of N metabolism during the incubation of organic residues under flooded and non-flooded conditions. These results suggest that biotic immobilization of fertilizer-N may play an important role in controlling N availability in paddy soils when crop residues are incorporated. Nevertheless, we cannot exclude that abiotic reactions between ammonia and lignin-phenols in the LF could have led to the formation of amide N directly bonded to aromatic rings (i.e. anilides) as reported by Schmidt-Rohr et al. (2004) and Olk et al. (2006) for humic acid fractions extracted from paddy soils. However, this mechanism is not expected to contribute significantly to N immobilization on the LF since this SOM fraction contains predominantly intact 

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Fig. 5. Total and fertilizer-derived (FDN) glucosamine-to-galactosamine N ratios for soils incubated under non-flooded (NF) or flooded (F) conditions, without (NS) or with (S) straw addition. The line within the box marks the median, the box boundary marks the 25th and 75th percentiles, whiskers above and below the box indicate the 90th and 10th percentiles.
lignin biopolymers with less phenolic hydroxyl functionalities with respect to lignin-derived constituents in the humic fractions. No significant amount of heterocyclic N structures, such as indoles, pyrroles and imidazoles, was detected in any of the LF samples analysed on the low field side of the main amide-peptide peak (between 120 and 213 ppm; Fig. 7), suggesting that their formation and contribution to immobilized N forms was limited within the experimental time frame.

4. Conclusions

The extent and mechanisms of N immobilization in paddy soils strongly depend on redox conditions and presence of labile OM. In fact, under flooded conditions 27–50% of applied N may be immobilized, with enhanced immobilization when rice straw is incorporated into the soil. This suggests that crop residue and water management practices adopted in rice cropping systems may strongly influence N immobilization and availability for the crop. In particular, the results obtained in this work give rise to the following implications:

1) Microbially-mediated processes were mainly responsible for the immobilization of applied N in both flooded and non-flooded paddy soils, particularly in the presence of labile OM. Most of the immobilized fertilizer-N was found to be associated with finer soil fractions, although significant amounts of applied N were also retained by the light fraction in those soils receiving straw. Microbial growth on decomposing straw seems to be mainly responsible for the transfer of applied N into particulate OM fractions. This provides further evidence that immobilization of applied N by soil microbes is intimately coupled with OM decomposition.

2) Under flooded conditions, the effective abiotic immobilization of applied N by interlayer NH\textsubscript{4}\textsuperscript{+} fixation may also play an important role in controlling N availability in relatively young, lowland rice paddies having a mineralogical composition rich in 2:1 clay minerals. We showed that under these conditions, NH\textsubscript{4}\textsuperscript{+} fixation may be responsible for the immobilization of 4–11% of applied N. Nevertheless, weakly fixed fertilizer-N may be made available during the cropping season, although its release is mainly regulated by biotic processes. Re-establishing oxic soil conditions during field drainage as well as N assimilation by the crop may influence the equilibria and release of fixed fertilizer-N. However, understanding the magnitude and dynamics of these processes as a function of plant N uptake during the cropping season still requires further research.

3) Soil redox conditions were shown to have a strong influence on aggregate stability. Moreover, results have evidenced that, apart from implications on OM stabilization, changes in aggregate stability with flooding may also affect biotic and abiotic processes controlling the immobilization and release of applied N.

Acknowledgements

Research was partly supported by the Italian Ministry of Agriculture, Food and Forestry (MiPAAF) under the project “Sustaining the National Rice Industry through Research, Technology, Innovation and Formation (POLORISO)”. D. Said-Pullicino acknowledges financial support from COST Action “Stable Isotopes in Biosphere-Atmosphere-Earth System Research (SIBAE)”. We are grateful to C. Minero for the use of his IRMS, M.R. Chierotti for his assistance with the NMR spectra, and S. Bösel and M. Dippold for their help with the compound-specific IRMS analysis.

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