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Nitrogen fertilizer fate after introducing maize and upland-rice into continuous paddy rice cropping systems

Irabella Fuhrmann[a,e], Yao He[a], Eva Lehndorff[a], Nicolas Brüggemann[b], Wulf Amelung[a], Reiner Wassmann[c,d], Jan Siemens[e,*]

[a] Institute of Crop Science and Resource Conservation (INRES) – Soil Science and Soil Ecology, University of Bonn, Nussallee 13, 53115 Bonn, Germany
[b] Forschungszentrum Jülich GmbH, Institute for Bio- and Geosciences, Agrosphere (IBG-3), Wilhelm-Johnen-Straße, 52428 Jülich, Germany
[c] Karlsruhe Institute of Technology (KIT), Institute of Meteorology and Climate Research Atmospheric Environmental Research (IMK-IFU), Kreuzeckbahnstraße 19, 82467 Garmisch-Partenkirchen, Germany
[d] Crop and Environmental Sciences Division, International Rice Research Institute, Los Baños, Philippines
[e] Institute of Soil Science and Soil Conservation, iFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35329 Giessen, Germany

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ABSTRACT

Water scarcity and economic incentives favor the introduction of upland crops into permanent paddy rice systems during dry seasons. However, introducing upland crops into permanently flooded cropping systems temporarily changes soil conditions from anaerobic to aerobic, affecting nitrogen (N) dynamics profoundly. We hypothesized that under maize and dry rice, total fertilizer ^{15}N recovery in soil as well as the immobilization of fertilizer ^{15}N in microbial residues is reduced compared with continuous paddy rice cropping. Furthermore, we expected enhanced emissions of fertilizer ^{15}N in form of nitrous oxide (N_2O) under maize and dry rice. To test these hypotheses, we traced the fate of a ^{15}N-urea pulse in a field experiment in the Philippines with three different crop rotations: continuous paddy rice, paddy rice – dry rice, and paddy rice – maize for two years. Indeed, the ^{15}N recovery in the first 5 cm of bulk soil was lowest in the paddy rice – maize rotation (arithmetic mean with standard error: 19.2 ± 1.8% of applied ^{15}N), while twice as much was recovered in the first 5 cm of bulk soil of the continuous paddy rice cropping systems (37.8 ± 2.2% of applied ^{15}N) during the first dry season. The ^{15}N recovery in the plant biomass (shoots and roots) in the continuous paddy rice cropping was 13% larger than in the dry rice plant biomass and 5% larger than in the maize plant biomass during the first dry season. Fertilizer ^{15}N remained longest in paddy rice – maize (mean residence time = 90 ± 25 days) and in continuous paddy rice (mean residence time = 77 ± 30 days), compared with dry rice – paddy rice rotation (mean residence time = 16 ± 5 days). After 2 years, 10% (paddy rice – dry rice, paddy rice – maize) to 23% (continuous paddy rice) of the applied fertilizer ^{15}N were still stored in soil. The largest fraction of this ^{15}N was immobilized by soil microbes, which stored 3–4% of applied ^{15}N in the form of amino sugars as specific cell wall constituents, in all cropping systems. Nevertheless, introducing upland crops into continuous paddy rice systems likely increased N leaching losses and resulted in initial losses of urea- ^{15}N to N_2O, which thus has to be considered in climate smart mitigation strategies.

1. Introduction

Rice (*Oryza sativa* L.) is one of the three most important food crops next to wheat and maize (FAO, 2016a). Worldwide, almost 165 million hectares (FAO, 2017) are used for rice production and 88% of rice plants are grown under flooded conditions (IRRI, 2012). However, water scarcity is an important issue in rice production, even though the irrigation water is predominantly re-used (Steduto et al., 2012). Paddy rice systems are therefore under change. Non-flooded crops, such as dry rice (also upland rice or aerobic rice), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), are more and more integrated into paddy rice cropping systems in dry seasons (Bouman et al., 2007; Timsina and Connor, 2001). Dry rice needs less water and nutrients compared to flooded rice (Gupta and O'Toole, 1986), but yields are comparably lower than those obtained with paddy rice (Belder et al., 2005). Maize, in turn, has the advantage that it also supplies livestock and poultry feed. In Southeast Asia, particularly the paddy rice − maize cropping system is thus increasingly used for food and fodder production

* Corresponding author.
E-mail address: jan.siemens@umwelt.uni-giessen.de (J. Siemens).

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(Timsina et al., 2010). The rotation of maize and paddy rice has recently been taken as an example for the "Save and Grow" strategy of the FAO for the sustainable intensification of cereal production (FAO, 2016b). Worldwide, rice and maize both currently contribute to 42.5% of world's food calory supply (FAO, 2016b).

The usage of nitrogen (N) fertilizer in paddy rice production is intensive. In 2004 the rate of N fertilization of rice (226 kg N ha^{-1}, Ma et al., 2008) exceeded N fertilization of maize by 5% and N fertilization of wheat by 12%. However, the N use efficiency is less than half of the efficiency typically found in upland crops (Kögel-Knabner et al., 2010; Olk et al., 1996). With the introduction of maize, there is an additional risk of N losses, particularly due to crack formation in the first years after maize production (He et al., 2017). Yet, the same authors also stated that with prolonged cropping duration, the system adapts and additional N leaching losses caused by maize decrease. However, to our knowledge a full N balance after introducing an upland crop into permanent paddy rice is still lacking.

Cassman et al. (2002) compared several experiments with maize, rice or wheat cultivation and found largest average fertilizer N recoveries for maize (37%), followed by rice (36%) and wheat (32%). In experiments dealing with rice – wheat crop rotations, rice plants used more soil organic N than wheat, for example (Shinde et al., 1985). Other experiments dealing with dry rice found lower fertilizer recoveries in aerobic rice plants and in the soil than in the permanently flooded fields (Belder et al., 2005; Kadiyala et al., 2015). Kadiyala et al. (2015) found larger recoveries in soil of the flooded rice after the first season, so that the following crop (in this case maize) could take up a larger amount of residual fertilizer N, suggesting that the fate of N can be traced beyond one cropping season. Cao and Yin (2015) found a similar pattern in a permanently flooded rice system. In their experiment, the major fraction of applied ^{15}N was also recovered in plant biomass, followed by the recovery in soil (0–20 cm). The rest was either lost via ammonia (NH_3) emissions or unaccounted.

Zhao et al. (2009) found 6% of the applied N in form of NH_3 in rice and 1% in wheat in a rice-wheat crop rotation. Large rates of nitrous oxide (N_2O) emissions were measured for the wheat crop, but also during fallow periods of the paddy rice crop, when aerobic-anaerobic cycles occurred with elevated amounts of soluble nitrate (NO_3^-, Pathak et al., 2002). This amount can be reduced when nitrogen is immobilized by microorganisms (Olk et al., 1996; Pande and Becker, 2003). Part of this microbial N is bound in amino sugars, which are specific markers for the residues of bacteria and fungi (e.g. Amelung et al., 2008; Murugan and Kumar, 2013; Said-Pullicino et al., 2014). Although amino sugar-N represents only a small portion of the microbially bound N, it accounted for up to 3.7% of total N in paddy and non-paddy surface soils (Roth et al., 2011). The fate of amino sugars is influenced by the supply of available C sources and N transformation processes and responds, thus, to changes in soil management and the resulting changes in soil properties (Amelung et al., 2001a; Ding et al., 2011; Lauer et al., 2011). Overall, 26 amino sugars have been recognized in microorganisms, four of them are detectable in soil. Glucosamine (Glu) is primarily a component of the chitin in the fungal cell walls, though it also can be found in some bacteria. Muramic acid (MurA) originates uniquely from bacterial cell wall residues. The origin of galactosamine (Gal) and mannosamine (Man) is less clear. It has been suggested that Gal is derived from bacterial genera, but other evidence suggests that it may also be derived from fungi (Amelung et al., 2008; Glaser et al., 2004; Liang and Balser, 2010). Appuhn and Joergensen (2006) suggested that the concentration of amino sugars may even serve as a proxy for the content of living biomass, using a conversion factor of 9 for fungi and of 45 or higher for bacteria when assuming that fungi and bacteria contain 46% C. However, the response of microbial residues to land-use change will be slower than for living biomass, particularly with an expected mean residence time of amino sugars in the range of a few years (Derrien and Amelung, 2011). Combining ^{15}N-fertilization with compound-specific ^{15}N analysis of these amino sugars may provide a clue to the in-situ turnover and sequestration rate of fertilizer N into the residues of both bacteria and fungi (He et al., 2006; Liang and Balser, 2010).

Analyses of N_2O emission in combination with ^{15}N-fertilization may help to elucidate the overall environmental impact of introducing upland crops into paddy rice systems. Pathak et al. (2002) indicated that NO_3^- served as a substrate for N_2O production and was predominantly produced in soils with changing soil moisture conditions, where denitrification and nitrification processes occur simultaneously. Although the contribution of N_2O emissions to total N loss and thus the reduction of nitrogen use efficiency is comparatively low, these emissions are critical for the greenhouse gas balance of the cropping system.

The objective of our study was to compare the utilization of fertilizer N in three rice dominated cropping systems: double paddy rice (R-Wet), paddy rice – dry rice (R-Dry) and paddy rice − maize (M-Mix) using ^{15}N-labelled urea as fertilizer. We hypothesize that the introduction of maize or dry rice results in:

(i) reduced ^{15}N fertilizer recoveries in bulk soil and plants for maize and dry rice,
(ii) larger immobilization of fertilizer ^{15}N in microbial residues for permanent paddy rice,
(iii) and larger emissions of fertilizer ^{15}N in form of N_2O for maize and dry rice,

compared to double paddy rice.

2. Materials and methods

2.1. Study site and experiment design

The field experiment was conducted at the experimental station of the German Research Foundation (DFG) research unit 1701 ICON at the International Rice Research Institute (IRRI) in Los Baños, Philippines (14° 11′ N, 121° 15′ E). The average long-term temperature in this area is 25.2 °C (1976–2011). In 2012 to 2014, the mean temperature was 27.5 °C. Annual precipitations were 2270 mm, 2350 mm and 1550 mm in the years 2012, 2013, and 2014, respectively, thus varying over the long-term average of 2006 mm per year. Most of the precipitation (67–78%) fell during the wet season (June–November), 22–33% during the dry season (December-May), respectively. Climate data were documented by the climate unit at IRRI. Detailed rainfall and temperature data can be accessed in the Supplementary data (Fig. S1). Soil properties were determined before the experiment started. The soil was developed from fluvial sediments overlying volcanic tuff and classified as Hydragric Anthrosol with clay dominated soil texture (Table S1) according to World Reference Base (IUSS Working Group WRB, 2015). The studied area has been at least 50 years under continuous paddy rice cultivation before the experiment started in 2011.

Three cropping systems were investigated with three replicates each: continuous paddy rice (R-Wet), paddy rice – dry rice (R-Dry), and paddy rice – maize (M-Mix). In separate fields we installed nine PVC rings (Ø 113 cm, 55 cm height) down to 25 cm soil depth (plough pan) to avoid the lateral loss of N to the rest of the field. The upper rim of the PVC ring extended 30 cm above the soil surface to prevent lateral exchange of irrigation water with the surrounding field (Fig. S2). Land preparation, irrigation, groundwater regulation and the drainage in the PVC rings were done manually. Weeds were uprooted and placed on top of the soil inside the ring. According to IRRI crop management routine, our field was fertilized with solophos (18% P) and potash (60% K) before seeding. We applied N in form of urea (46% N). Rice (both paddy rice and dry rice) received a total fertilization of 130 kg N ha^{-1}, in three splits of 30, 50, 50 kg N ha^{-1} (3, 5, 5 g N $ring^{-1}$). Maize was fertilized with 60, 30, 60 kg N ha^{-1} (6, 3, 6 g N $ring^{-1}$). The ^{15}N labelling for the main experiment was conducted as the first N split in the 2012 dry season with ^{15}N-urea (95 atom-%, Campro Scientific GmbH,

Veendendaal, Netherlands) in all treatments (R-Wet: 16 February 2012, R-Dry and M-Mix: 28 February 2012). The ^{15}N-urea was dissolved in water and sprayed on the soil surface to ensure an even spatial distribution. For the wet season a second experiment with only two treatments (R-Wet and M-Mix) was conducted for N_2O gas measurements. The ^{15}N-labelling for this experiment was applied on 18 August 2012 with the same labelled fertilizer

2.2. Soil and plant sampling

During the experimental period, bulk soil samples in the PVC rings were collected at 0–5 cm and 5–20 cm before each fertilization and before harvest. Each soil sample was separated into two aliquots. One aliquot was stored at 4 °C for the NH_4^+ and NO_3^- (inorganic N) extraction at IRRI immediately after sampling, while the other aliquot was frozen at −18 °C. The frozen soil samples were freeze-dried, sieved to ≤2 mm and milled for analysis of total N content and δ^{15}N signature in Germany.

The aboveground biomass (shoots) and roots with rhizosphere soil were collected during tillering, panicle formation, flowering and maturity, respectively. Shoots taken during maturity were separated from corncobs and rice grains. Roots with rhizosphere soil were frozen at −18 °C immediately after sampling for the transport to Germany. In Germany, these samples were defrosted gently at 4 °C. The rhizosphere soil was separated from roots using the method of He et al. (2015). Shoots and washed roots were oven-dried at 75 °C and milled. Total numbers and types of analysed samples are listed in Table S2.

2.3. N_2O gas sampling

In the dry season, a 0.01 m^3 chamber (Ø 28.5 cm) was placed for 16 h (4 p.m. to 8 a.m.) in each PVC ring prior to the sampling. In the last half hour, air inside the chamber was homogenized with a 12 V ventilator. Gas samples with a total volume of 500 mL were collected with a syringe from each chamber and flushed through 100-mL glass vials sealed with a septum, using a second needle as outlet. After removal of the outlet syringe, a final sample volume of 125 mL was pressed into the 100 mL glass vials using the syringe to establish an excess pressure that prevented the inflow of atmospheric air.

During the wet season, the gas sampling method was modified for the second experiment. Bigger chambers (0.6 m^3) and shorter closing time of the chamber were used to reduce the accumulation of ^{15}N-labelled N_2O inside the chamber. A 12 V ventilator homogenized the air inside the chamber. One hour after closing the chamber, the gas samples were collected, with the same collecting and flushing method as in the dry season.

Gas samples were taken at day 0, 1, 2, 4 and 7 after ^{15}N labelling in all PVC rings in the dry and wet season.

2.4. Nitrogen analyses

For the extraction of exchangeable ammonium (NH_4^+) and NO_3^-, 20 g fresh soil was mixed with 100 mL 2 M KCl for 1 h before it was filtered (ashless paper, no. 40, Whatman GmbH, Dassel, Germany). The extractions were analysed in the analytical service laboratory (ASL) of IRRI by colorimetric analysis. The soil extractable NH_4^+-N and NO_3^--N (inorganic N) were investigated for the year 2012, in which the labelled N fertilizer was applied.

The total N contents and δ^{15}N signature of bulk soil, shoots, and roots were determined with an elemental analyser (Flash EA1112, ThermoFisher Scientific GmbH, Bremen, Germany) coupled with an isotope ratio mass spectrometer (EA-IRMS, Delta V Advantage, ThermoFisher Scientfic GmbH, Bremen, Germany). The amount of analysed samples per season are listed in Table S2. The stable N isotope signatures of the samples were expressed as δ^{15}N in per mill as calculated in relation to atmospheric N_2 ($\delta^{15}N_{atm}$) and ammonium nitrate-$\delta^{15}N_2$ standard (98.23 atom-%, Campro Scientific GmbH, Veendendaal, Netherlands). To assess the excess ^{15}N derived from the ^{15}N-labelled fertilizer, Eqs. (1) and (2) were used (Epron et al., 2011):

$$Ab = \frac{^{15}N}{^{14}N + ^{15}N} = \frac{(\frac{\delta^{15}N}{1000} + 1)*\delta^{15}N_{atm}}{[(\frac{\delta^{15}N}{1000} + 1)*\delta^{15}N_{atm}] + 1} \quad (1)$$

$$^{15}N_{excess} = (Ab - Ab_{unlabelled}) \times 100 \quad (2)$$

with Ab denoting the relative abundance of ^{15}N in a sample, $Ab_{unlabelled}$ as relative ^{15}N abundance in the unlabelled control samples that were collected before ^{15}N labelling. References for plant biomass ^{15}N abundance were taken from Handley and Raven (1992). The recovery of ^{15}N ($^{15}N_{recovery}$) was calculated after Eq. (3):

$$15_{N_{recovery}} = \frac{15_{N_{excess} \times material\ amount}}{15_{N_{applied}}} \times 100 \quad (3)$$

With material amount as the amount of the analysed soil (per m^2 in the sampled depth), plant or root amount (dried plant material per m^2) in the labelled area and $^{15}N_{applied}$ as the applied fertilizer ^{15}N.

2.5. Amino sugar analysis

Topsoil samples (0–5 cm) of nine sampling dates were chosen for amino sugar analysis out of which seven were taken throughout 2012 and the others in 2013 and 2014.

The amino sugar analysis was conducted according to the protocol of Zhang et al. (1997). Amino sugars were extracted from 150 to 200 mg soil, corresponding to 0.3 mg N per sample, using 10 mL of 6 M HCl and 100 µL of internal recovery standard (IS1: 1 µg *myo*-inositol µL^{-1} 0.1 M HCl; 100 µg per sample) were added and hydrolyzed (HCl) for 8 h at 105 °C. After cooling, the samples were filtrated (glass fiber filters, GF 6, Whatman GmbH, Dassel, Germany). The filtrate was concentrated with a rotary evaporator at 1.5 MPa and 40 °C (Büchi Synchore Polyvap (R-12); Büchi Labor Technik GmbH, Essen, Germany). After dissolving the residues with Millipore water, the pH was adjusted to pH 6.6–6.8 and centrifuged (1500*g* for 10 min). The clear supernatant was freeze-dried and diluted 3 times with 1.5 mL methanol. After centrifugation the supernatant was transferred into reactivials, and then evaporated with a stream of pure nitrogen (> 99.9 n/n%). Finally, it was dissolved with 1 mL Millipore water and freeze-dried. The hydrolysates were dissolved in 300 µL derivatization reagent containing 360 mg hydroxylamine hydrochloride, 450 mg 4-(dimethylamino)-pyridin, 9 mL pyridin and 2.25 mL methanol. The samples were derivatized for 30 min at 80 °C (oil bath). After that, 1 mL of acetic anhydride was added and derivatized again for 20 min. 1700 µL dichloromethane was added to the reactivials, which were then shaken. For the washing step, 1 mL 1 M HCl was added to the reactivials and shaken for 10 s. The liquid phase was removed. The washing step was repeated 3 times with 1 mL Millipore water. Afterwards, 100 µL of internal quantification standard (IS2: 1 µg ß-endosulfan µL^{-1} methanol = 100 µg per sample) was added to the reactivials. The solvents were evaporated with a stream of pure nitrogen (> 99.9 n/n%). The residue was dissolved with 300 µL ethyl acetate/hexane (1:1 v/v) and transferred into crimp neck vials.

External standard series were prepared with concentration levels of 20, 40, 80, 100, 200, 300, 400 µg of MurA, 100 µg of Glu, Man, Gal and 100 µL of IS1 and IS2 to confirm linearity of the detection method for MurA. External standards with 100 µL of MurA, Glu, Man, Gal and 100 µL of IS1 and IS2 were prepared for each run to determine the response factor for amino sugar derivatives, related to the recovery of IS1. The concentration of detected amino sugars was related to the response factor. The recovery of detected amino sugar derivatives were related to IS1 in each sample.

Determination of amino sugar derivatives was done by gas chromatography-mass spectrometry (GC–MS 6890N, Agilent Technology,

California, USA) with column OPTIMA®5 MS (Machery-Nagel, Düren, Germany). Helium was used as a carrier gas with a flow rate set at 1.1 mL min^{-1} at 0.8 bar. The split ratio was 30:1. The initial temperature of the column was 120 °C, held for 1 min. Then the temperature increased at a rate of 10 °C min^{-1} to 250 °C and at a rate of 20 °C min^{-1} to 270 °C. The injector and detector temperatures were 250 °C and 300 °C, respectively. The injected volume was 2 µL.

The mass spectrometer was operated in electron ionization (EI) and selected ion monitoring (SIM) mode looking at mass-to-charge ratios (m/z) of 89, 99, 236, 237, 264, 265 and 356, 357 to identify the ^{15}N label in amino sugars (according to He et al., 2006).

^{15}N isotope signatures were expressed as the abundance ratio (R) of the ^{15}N-labelled amino sugar fragments versus the abundance of the unlabelled amino sugar fragments (F = unlabelled, and F + 1 = labelled fragment).

$$R = \frac{F+1}{F} \tag{4}$$

In addition, ^{15}N atom percentage excess (APE) was calculated according to (He et al., 2006):

$$\mathrm{APE} = \frac{R_e - R_c}{(R_e - R_c) + 1} \times 100 \tag{5}$$

where R_e is the enriched ratio of ^{15}N/^{14}N in a labelled sample, R_c is the corresponding ratio obtained from external standards (control) analysed in the same GC–MS run, F are the m/z ratios for Glu (98), Gal (236), Man (264) and for MurA (356) (He et al., 2006). The excess of ^{15}N in the amino sugars can be calculated after He et al. (2011):

$$15_{N_{\mathrm{excess}}} = \frac{\mathrm{APE}}{100} \times amino\ \ sugar - N_{\mathrm{content}} \tag{6}$$

Where amino sugar $N_{content}$ is the content of N in the respective amino sugar. ^{15}N recovery in amino sugars was calculated according to Eq. (3).

2.6. δ^{15}-N_2O analysis

Gas samples were analysed for δ^{15}-N_2O by using an isotope ratio mass spectrometer (IsoPrime 100, Elementar Analysensysteme, Hanau, Germany), which was equipped with a pre-concentration unit (TraceGas, Elementar Analysensysteme) for online separation and purification of N_2O. Isotope values of N are reported in the delta notation, with:

$$\delta = \frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}} - 1} \times 100 \tag{7}$$

Where R_{sample} and $R_{standard}$ are the ratios of ^{15}N to ^{14}N in the sample and in the atmosphere ($\delta^{15}N_{atm}$). The measuring range of δ^{15}-N_2O covered up to 25 000‰.

2.7. Statistical evaluation

All statistical analyses were performed with SPSS 21.0 (SPSS, Inc., USA). The assumptions for normality and homogeneity of variance were tested with the Shapiro-Wilk test. Differences between the cropping systems or between amino sugars were tested by one-way analysis of variance (ANOVA) and the LSD method at a 95% confidence level, unpaired *t*-test or Kruskall-Wallis test. Differences between the seasons or soil depths were tested by repeated measurements ANOVA and paired *t*-test or Wilcoxon test. The Pearson correlation coefficient was used to analyse the bivariate correlation between amino sugar and N_{tot}. For creating all graphs, as well as for determining the mean residence times (MRTs) of fertilizer ^{15}N, Sigmaplot 12.5 (Systat Software GmbH, Erkrath, Germany) was used.

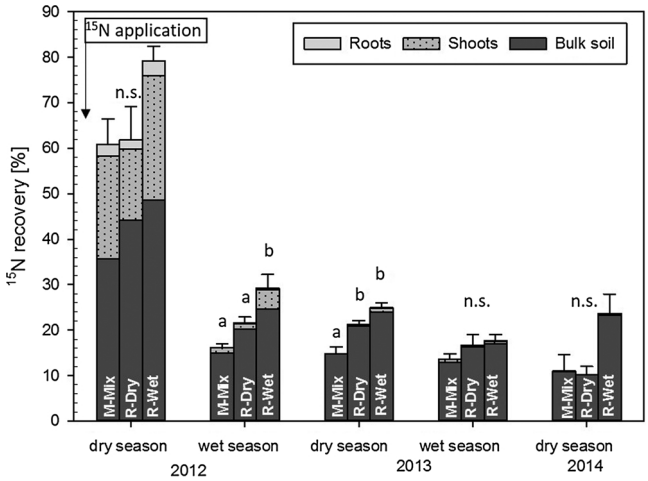

Fig. 1. Recovery of fertilizer-^{15}N (%) in topsoil (0–20 cm), shoots and roots of all cropping systems (maize – paddy rice (M-Mix), dry rice – paddy rice (R–Dry), paddy rice – paddy rice (R–Wet)) in 2012–2014 (error bars = SE of sum of recoveries in soil, shoot and roots samples, n = 3). Shoot samples from M-Mix in dry season 2013 are not available. Different case letters indicate significant differences within the varieties of the respective period ($p < 0.05$, n.s. not significant).

3. Results

3.1. Fertilizer N recovery in plant tissues

As calculation of excess ^{15}N in plant biomass is not only reflecting fertilizer use efficiency but depends also on shoot biomass and amount of fertilizer applied, we calculated the recovery of the added fertilizer N in the respective plant material. Data of excess ^{15}N can be assessed in the Supporting information (Table S4).

In the shoots, we found the largest average recovery of fertilizer ^{15}N during the first dry season of the R-Wet rotation, which did not differ significantly from those of permanent rice in M-Mix, yet exceeding the recovery in dry rice ($p < 0.05$, Fig. 1). On the first sampling date, only 23 days after ^{15}N application, 48% of the fertilizer could be recovered in the shoots of R-Wet, whereas the largest values in M-Mix were reached 57 days after ^{15}N application (38%). For R-Dry, the recovery of ^{15}N in shoots remained around 16–17% before dropping to 11% during harvest in 2012 (97 days after ^{15}N application). During the following wet season, the rice shoots of the R-Wet rotation captured the largest fraction of applied fertilizer N (Fig. 1, $p < 0.05$), while similar amounts of applied ^{15}N were recovered in rice shoots of the R-Dry and in maize shoots of the M-Mix rotation (Fig. 1, n.s.). The ^{15}N fertilizer label was still detectable in the in shoots even two years after the application in the dry season, though only at less than 1% of the originally applied amounts and with no difference between crop rotations.

The largest average recovery of applied ^{15}N in roots was found in R-Wet during the first dry season after ^{15}N application (3.2 ± 0.03%), followed by M-Mix (2.6 ± 0.04%, n.s.) and R-Dry (2.0 ± 0.08%, $p < 0.05$). In the following seasons (wet season 2012 to dry season 2014) the recoveries in all cropping systems were significantly smaller (Fig. 1, $p < 0.005$). Only in the first wet season after ^{15}N application, the fertilizer could be traced in roots, with even lower portions than in the shoots: the portions of ^{15}N fertilizer captured by the roots then declined in the order roots in R-Wet (0.3% of applied amount) > R-Dry (0.2%) > M-Mix (0.1%; $p < 0.05$). After the first wet season, fertilizer ^{15}N recovery in the roots in all cropping systems remained below 0.06%.

3.2. Fertilizer N recovery and fate in soil

The total N content of bulk soil was not significantly influenced by the crop management over the experimental period of 2 years (0–20 cm,

Table 1
Contents of total N (N_{tot}), NH_4^+-N, NO_3^--N in soil (0–5 cm and 5–20 cm) and amino sugar-N content in topsoil (0–5 cm) of maize − paddy rice (M-Mix), dry rice – paddy rice (R-Dry), and paddy rice – paddy rice (R-Wet), Values denote the arithmetic mean ± standard error (n = 3).

Variet	N_{tot}								NH_4^+-N								NO_3^--N								Amino sugar-N			
	[mg kg^{-1} soil]								[mg kg^{-1} soil]								[mg kg^{-1} soil]								[mg kg^{-1} soil]			
	0–5 cm				5–20 cm				0–5 cm				5–20 cm				0–5 cm				5–20 cm				0–5 cm			
	2012 dry season																											
M-Mix	1885	±	60	ab[a]	1608	±	54	n.s.	1.26	±	0.09	a	1.16	±	0.05	a	3.53	±	0.73	n.s.	1.22	±	0.35	a	124	±	6	a
R-Dry	1777	±	56	a	1602	±	13	n.s.	1.55	±	0.40	ab	1.06	±	0.23	a	1.96	±	0.25	n.s.	1.15	±	0.18	a	73	±	6	b
R-Wet	2084	±	28	b	1544	±	36	n.s.	11.80	±	4.72	b	2.13	±	0.35	b	0.03	±	0.00	n.s.	0.04	±	0.01	b	143	±	7	a
	2012 wet season																											
M-Mix	1801	±	47	n.s.	1470	±	74	n.s.	2.17	±	0.09	n.s.	1.69	±	0.04	n.s.	0.17	±	0.02	n.s.	0.30	±	0.03	n.s.	140	±	14	a
R-Dry	1769	±	24	n.s.	1519	±	45	n.s.	2.57	±	0.20	n.s.	1.85	±	0.10	n.s.	0.15	±	0.00	n.s.	0.34	±	0.03	n.s.	66	±	11	ab
R-Wet	1859	±	32	n.s.	1406	±	61	n.s.	2.89	±	0.19	n.s.	1.90	±	0.08	n.s.	0.17	±	0.01	n.s.	0.23	±	0.02	n.s.	75	±	6	b
	2013 dry season																											
M-Mix	*1640*	±	59	n.s.	1373	±	22	n.s.	n.a.				n.a.				n.a.				n.a.				162	±	24	n.s.
R-Dry	1689	±	72	n.s.	1426	±	27	n.s.																	180	±	3	n.s.
R-Wet	1669	±	43	n.s.	1411	±	31	n.s.																	149	±	8	n.s.
	2013 wet season																											
M-Mix	1629	±	61	n.s.	1379	±	83	n.s.	n.a.				n.a.				n.a.				n.a.				n.a.			
R-Dry	1709	±	35	n.s.	1419	±	43	n.s.																				
R-Wet	1615	±	20	n.s.	1402	±	118	n.s.																				
	2014 dry season																											
M-Mix	1506	±	24	n.s.	1132	±	318	n.s.	n.a.				n.a.				n.a.				n.a.				143	±	27	n.s.
R-Dry	1627	±	25	n.s.	1408	±	74	n.s.																	126	±	6	n.s.
R-Wet	1638	±	57	n.s.	1393	±	111	n.s.																	134	±	9	n.s.

[a] Different case letters indicate significant differences between different crop rotations within the respective season ($p < 0.05$, n.s. not significant, n.a. data not available).

Table 1). In all variants, total N contents (N_{tot}) were larger in the surface soil (0–5 cm) than in deeper soil layers (5–20 cm), despite regular soil mixing by puddling and/or ploughing (Table 1, $p < 0.05$).

Inorganic N contributed less than 0.2% to total N stocks in all treatments during the first dry season. In addition, the R-Wet trials maintained significantly smaller contents of NO_3^--N than of NH_4^+ during this season (Table 1, $p < 0.05$). Also in the first wet season, the dominant inorganic N form was NH_4^+-N in all treatments.

In all crop rotations, inorganic N concentrations of the deeper soil layers (5-20 cm) were smaller than for the uppermost 5 cm of the soil ($p < 0.005$) in 2012, also here mainly due to differences in NH_4^+-N concentrations (Table 1). Again, we found significantly larger NH_4^+-N and inorganic N concentrations for the R-Wet rotation compared to the other crop rotations also for the 5–20 cm soil depth interval (Table 1, $p < 0.05$). Intriguingly, NO_3^--N concentrations were larger at this depth interval than in the first 5 cm during the wet season (Table 1).

The recovery of fertilizer ^{15}N in soil decreased exponentially with time (Fig. 2, fitting parameters in Table S 5). The MRTs for the cumulative ^{15}N recoveries in 0–20 cm depth derived for M-Mix (90 ± 25 days) and R-Wet (77 ± 30 days) were larger than the MRT estimated for R-Dry (16 ± 5 days). During the first dry season after ^{15}N application, the cumulative ^{15}N recovery in 0–20 cm soil depth of R-Wet was 27% larger than in M-Mix and 9% larger than in R-Dry (Fig. 1). In the following wet season, the ^{15}N recovery in all treatments declined by roughly 50%. In comparison to the ^{15}N recovery in the plant material, the ^{15}N recovery in the bulk soil decreased less in 2013 and 2014, so that we could still find 23% of the applied fertilizer ^{15}N in R-Wet and roughly 10% in M-Mix and R-Dry at the end of the experiment (Fig. 1).

The decrease of the ^{15}N recovery was more pronounced for the top 5 cm of the soil compared to 5–20 cm soil depth (Figs. 2, S4). Differences between ^{15}N recoveries after the application of the labelled fertilizer in the first dry season for the R-Dry and M-Mix rotations in the top 5 cm of soil were larger compared to the differences in 5–20 cm depth. While similar MRTs of fertilizer ^{15}N of 90 ± 18 days and of 97 ± 29 days were derived for 0–5 cm soil depth of M-Mix and R-Wet, respectively, a much smaller MRT of 19 ± 2 days was estimated for R-Dry (Fig. 3a, fitting parameters in Table S5). In contrast to M-Mix and R-Dry, an increasing ^{15}N recovery for the 5–20 cm depth of R-Wet was

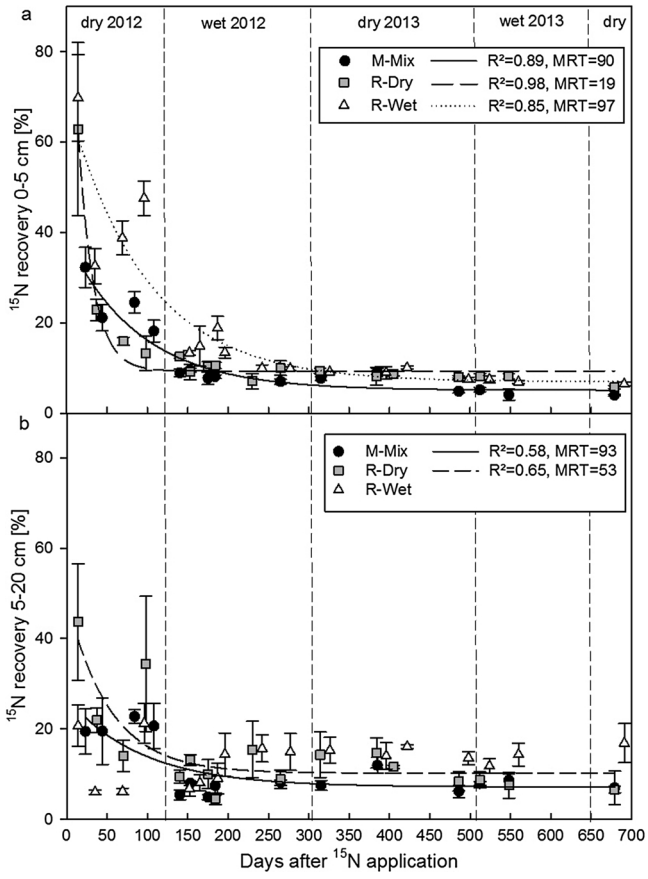

Fig. 2. Recovery of ^{15}N in topsoil (a: 0–5 cm, b: 5–20 cm) of all cropping systems (maize – paddy rice (M-Mix), dry rice – paddy rice (R-Dry), paddy rice – paddy rice (R-Wet)) in 2012–2014 (error bars = SE, n = 3). Lines represent different mean residence times (MRT) of each cropping system with $^{15}N_{recovery} = a \times e\,(-b \times x)$. The equation did not fit to the values of R-Wet (5–20 cm) because of the increasing values ($R^2 < 0.2$).

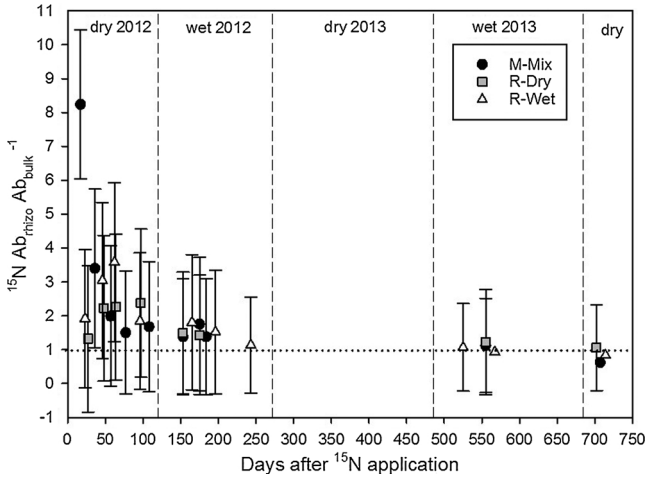

Fig. 3. Geometric mean of the ratio between ^{15}N abundance (Ab) in the rhizosphere (Abrhizo) and the Ab in bulk soil (Ab_{bulk}) of all cropping systems (maize – paddy rice (M-Mix), dry rice – paddy rice (R-Dry), paddy rice – paddy rice (R-Wet)) in 2012–2014 (error bars = SE, n = 3). Samples from dry season 2013 are not available.

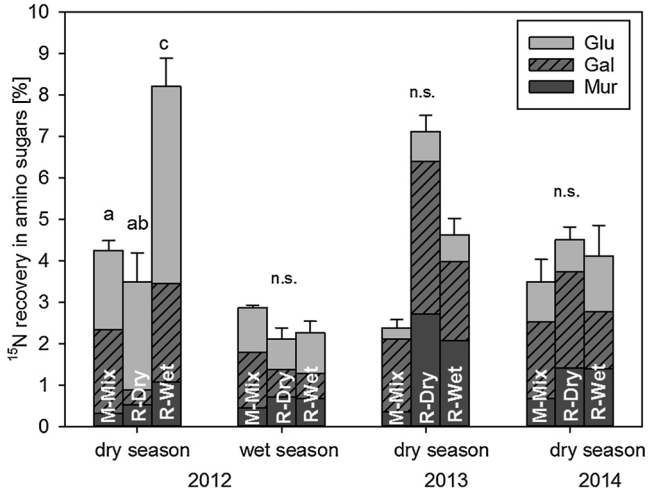

Fig. 4. Recovery of fertilizer ^{15}N (%) in amino sugars glucosamine (Glu), galactosamine (Gal) and muramic acid (Mur) in topsoil (0–5 cm) of maize – paddy rice (M-Mix), dry rice – paddy rice (R-Dry), and paddy rice – paddy rice (R-Wet) cropping systems in 2012 to 2014. Values denote the arithmetic mean ± standard error (n = 3). Different case letters indicate significant differences within the varieties of the respective period ($p < 0.05$, n.s. not significant).

observed two years later, which prevented the calculation of a MRT using the exponential dissipation model. For M-Mix, the MRT estimated for 5–20 cm depth (93 ± 56 days) resembled the one derived for 0–5 cm soil depth. For R-Dry, a larger MRT of 53 ± 23 days was estimated for 5–20 cm depth than for the 0–5 cm depth increment (Fig. 2).

It was not possible to convert the ^{15}N excess into a recovery of applied fertilizer ^{15}N in the rhizosphere because of the poorly defined dimension and thus mass of rhizosphere soil. Instead of comparing ^{15}N recoveries, we therefore calculated the ratio of ^{15}N abundance in rhizosphere soil and in the bulk soil to assess differences in ^{15}N dynamics between rhizosphere and bulk soil (Fig. 3). This ratio is independent of the different amounts of fertilizer applied to the three crops. The distribution of fertilizer ^{15}N between rhizosphere and bulk soil during the first dry season after ^{15}N application differed significantly from the following seasons for all cropping systems ($p < 0.05$). Ratios larger than one ($p < 0.05$) indicated a preferential accumulation of fertilizer ^{15}N in rhizosphere soil compared to bulk soil in all crop rotations in the dry season 2012 until the dry season 2013, i.e., about 200 days after ^{15}N fertilizer application. During this time the distribution of ^{15}N between rhizosphere and bulk soil was strongly affected by the cropping system: under maize, the rhizosphere soil contained eight times more ^{15}N than the bulk soil directly after the application of fertilizer ^{15}N in the dry season 2012 (Fig. 2). This strong enrichment of ^{15}N in the rhizosphere was significantly larger than that in the rhizosphere of rice in the R-Wet and R-Dry rotations ($p < 0.05$). With time, the gradients disappeared exponentially as did total fertilizer recovery; yet, also in the second wet season 2013 the ratio was significantly larger than for M-Mix and R-Wet ($p < 0.05$), but not for the R-Dry trials. Two years after the ^{15}N application, the ratio for M-Mix and R-Wet decreased below 1, whereas the ratio for R-Dry maintained around 1.

3.3. ^{15}N in soil microbial residues

Amino sugars were used as biomarkers for microbial residues in the top soil layer. The largest average content of amino sugars was found for R-Wet (1.72 ± 0.06 g kg^{-1} soil), followed by M-Mix (1.68 ± 0.13 g kg^{-1}soil) and R-Dry (1.15 ± 0.04 g kg^{-1} soil). The amino sugar-N content correlated positively with the total N content ($r = 0.95$, $p = 0.01$). The amino sugar-N fraction of total N was largest in M-Mix (7.2 ± 0.4% of total N), followed by R-Wet (7.0 ± 0.2% of total N, n.s.) and R-Dry (5.1 ± 0.1% of total N, $p < 0.01$).

Among different amino sugars, Glu contained most soil N (3.9% of soil N), followed by Gal (2.4% of soil N), Man (0.1% of soil N), and MurA (0.03% of soil N) across all cropping systems. The soil under R-Dry had a significantly lower content of Glu-N (2.9% of total soil N) than the other two cropping systems (M-Mix: 4.5% of total soil N, R-Wet: 4.3% of total soil N, $p < 0.005$).

Across all crop rotations, most of the applied fertilizer ^{15}N was recovered in Glu (53–82% of the total ^{15}N recovery in amino sugars), followed by Gal (18-45% of the total ^{15}N recovery in amino sugars), and MurA (1–2% of the total ^{15}N recovery in amino sugars, Fig. 4, $p < 0.005$). Most of the ^{15}N could be recovered in amino sugars in R-Wet (8.2 ± 0.7% of the total ^{15}N recovery) during the first dry season, followed by M-Mix (4.3 ± 0.2% of the total ^{15}N recovery) and R-Dry (3.5 ± 0.7%, $p < 0.05$ of the total ^{15}N recovery). After the first season the differences between the cropping systems decreased. Moreover, the total ^{15}N recovery in amino sugars did not decrease further; yet, the proportions of ^{15}N initially found in Glucosamine declined over time relative to the proportions found in the other amino sugars (Fig. 4).

3.4. ^{15}N in N_2O

During the dry season 2012, the loss of applied ^{15}N in the form of $^{15}N_2O$ increased after labelling in all cropping systems (Table 2, $p < 0.001$). Comparing all cropping systems in the dry season 2012, R-Wet showed lower $\delta^{15}N$ values in emitted N_2O than M-Mix and R-Dry ($p < 0.005$). During the following wet season 2012, the loss of applied ^{15}N emitted in form of $\delta^{15}N$ in N_2O was again larger in M-Mix than in R-Wet ($p < 0.05$).

4. Discussion

4.1. Short-term N fate after introducing upland crops

The uptake of fertilizer N by wet rice may be accelerated compared to maize and dry rice. Wet rice was shown to consume NH_4^+ from the waterlogged soil, while dry rice and maize consume NO_3^- from the drained soils (Sasakawa and Yamamoto, 1978). Since NH_4^+ is more rapidly formed from the used fertilizer (urea, Smith and Dilday, 2003), we assumed most efficient N uptake by the flooded rice plants. In our experiment, the maize plants showed larger ^{15}N abundances, but in relation to the applied fertilizer amount, the recovery was larger in the rice plants of the R-Wet rotation. This was mainly due to smaller harvested maize biomass during the first maize plant samplings compared

Table 2
$\delta^{15}N$ in N_2O in gas samples of all cropping systems (maize – paddy rice (M-Mix), dry rice – paddy rice (R-Dry), paddy rice – paddy rice (R-Wet)), ± standard error (n = 3).

Days after ^{15}N application[a]		$\delta^{15}N_2O$ (‰)											
		M-Mix				R-Dry				R-Wet			
		2012 dry season											
0	A[b]	28	±	8	a[c]	−0.01	±	0.73	b	4.3	±	0.2	ab
1	B	21711	±	7237	a	> 25000			a	787	±	163	b
2	BC	19731	±	6958	a	> 25000			a	1580	±	478	b
4	DE	> 25000			n.s.	> 25000			n.s.	14805	±	5950	n.s.
7	BCD	16566	±	5522	n.s.	> 25000			n.s.	13077	±	4356	n.s.
		2012 wet season											
0	A	2.1	±	0.7	n.s.	n.a.				−0.3	±	1.1	n.s.
1	B	4241	±	1291	a					31.1	±	7.1	b
2	AC	5.9	±	2.6	a					3.0	±	0.4	b
4	C	2.6	±	1.4	a					3.8	±	0.2	b
7	C	4.0	±	0.1	n.s.					3.8	±	0.5	n.s.

[a] Application of ^{15}N-Urea in dry season: 13.04 g m^{-2} (M-Mix), 6.52 g m^{-2} (R-Dry, R-Wet), application of ^{15}N-Urea wet season: 6.52 g m^{-2} (M-Mix, R-Wet) as the first of three urea applications for the respective seasons.
[b] Different capital letters indicate significant differences between the sampling days within the respective season.
[c] Different lowercase case letters indicate significant differences between different crop rotations within the respective sampling day ($p < 0.05$, n.s. not significant, n.a. data not available).

to rice plant sampling. The first fertilizer split was applied with the maize seedlings, whereas the fertilizer application for rice was applied around two weeks after rice seeding. The later application of fertilizer N to rice may have increased the efficiency of ^{15}N uptake of rice plants compared to maize plants. In addition to the timing of fertilizer application, also the fate of fertilizer N in soil controls its uptake into plants and vice versa. Belder et al. (2005) hypothesized that the smaller ^{15}N recovery in a cropping system with irrigated aerobic rice compared to paddy rice was caused by larger gaseous losses in the system with aerobic rice.

Redox cycles alter the biogeochemical cycling of nutrients (e.g., Kögel-Knabner et al., 2010; Scalenghe et al., 2012). Here, fertilizer N could be lost to the gas phase or to the water phase (Janssen and Lennartz, 2007; Pathak et al., 2002; Zhao et al., 2009; Zou et al., 2005). These losses can either be detected by analyses of emitted soil air or leached soil water, or estimated from the portion of N we could not detect in plant and soil (=unaccounted fraction). We observed largest unaccounted fertilizer fractions in M-Mix and R-Dry (39%) in the dry season 2012.

In our case, the ^{15}N proportion in the rhizosphere may be part of the unaccounted fraction, because the recovery could not be quantified. In the longer-term, the rhizosphere N probably diffuses to the bulk soil, is taken up by the plant, or is lost via gaseous emissions. For M-Mix and R-Dry larger volatilization losses via mineralization/denitrification processes due to changes from wet to dry soil conditions were possible (Cai et al., 1997; Reddy and Patrick, 1974). Although large losses via NH_3 are probably negligible in the non-flooded periods of our experiment (De Datta, 1981), NH_3 losses can be enhanced after urea application due to increasing pH of the floodwater (Pan et al., 2016), which was not measured in this experiment, but can differ to the relatively low pH level in the soil solution ($pH < 6.8$) in our experiment.

In addition to gaseous losses, N leaching may strongly affect the recovery of fertilizer N. The drying of clay-dominated soils during upland cropping, such as growing dry rice or maize, causes unwanted water losses in consequence of crack formation (He et al., 2017; Witt et al., 2000; Zhao et al., 2009;). Witt et al. (2000) reported that in their experiments cracks extended only down to 5 cm depth. Due to larger clay contents of the soil at the IRRI, there were probably deeper soil cracks (up to 25 cm) not only in maize, but also in dry rice soils, which cause a large risk of leaching in both systems. Losses of fertilizer N in M-Mix and R-Dry prevent ^{15}N sequestration especially during the dry season, when the fertilizer is applied to drained soil. Reduced fertilizer recoveries in R-Dry and especially M-Mix indicated N-fertilizer losses directly after application and after the dry season 2012. It is likely that a significant fraction of the fertilizer ^{15}N in our experiment was directly leached from the soil, since He et al. (2017) found high leaching losses of 6.8 g N m^{-2} from the M-Mix rotation 2012, the first season after introducing maize into a continuous paddy rice system. Due to slower nitrification rates in the mainly flooded fields of R-Wet and an intact plough pan, there is a smaller risk of NO_3^--leaching than in R-Dry and M-Mix (Janssen and Lennartz, 2007; Lennartz et al., 2009; Zhu et al., 1989).

4.2. Longer-term N fate after introducing upland crops

Portions of fertilizer N may be stored in soil, depending on aerobic or anaerobic conditions. In our experiment, we could observe a partial retention of ^{15}N in 0–5 cm depth in R-Wet during the dry season 2012, leading to larger ^{15}N recoveries in shoot and root biomass in the following wet season.

Anaerobic conditions may have led to immobilization processes of N into SON. After soil preparation for the wet season 2012, N mineralization processes in the upper soil layers could have produced N that was available for the rice plants again. In our experiment this process can be seen by higher NH_4^+-N concentrations in M-Mix and R-Dry soils, and lower NO_3^--N in soils of all cropping systems during the wet season, compared to the first dry season. An accumulation of N in the topsoil under anaerobic conditions, which is then available for the following non-flooded crop was also observed by Belder et al. (2005) and Kadiyala et al. (2015). In line with Kadiyala et al. (2015) we found small recoveries of fertilizer ^{15}N in the following 3rd and 4th growing season. Higher abundances in the rhizosphere confirmed that the portions of plant available ^{15}N were largest in the first two seasons. After the first two seasons the fertilizer N was probably not plant-available anymore (Legg et al., 1971; Olk et al., 1996).

The low concentrations and fractions of inorganic N in all treatments indicate an effective uptake of N by crops (Masclaux-Daubresse et al., 2010) and a fast immobilization of inorganic N in organic forms (Roth et al., 2011). Due to plant uptake and immobilization, most of the N found in the soil is in form of SON (Pande and Becker, 2003; Zhu et al., 1989). In our experiment, the fertilizer N taken up by the aboveground plant material was removed from the field and was thus not available for the following cropping season.

Abiotic immobilization in continuous paddy systems can be a decisive factor. Low availability of oxygen and large clay contents of soils can cause higher concentrations of phenols (Olk et al., 1996). In our experiment fertilizer N could have been immobilized in this fraction. Consequently this part is protected against leaching, but also not plant-

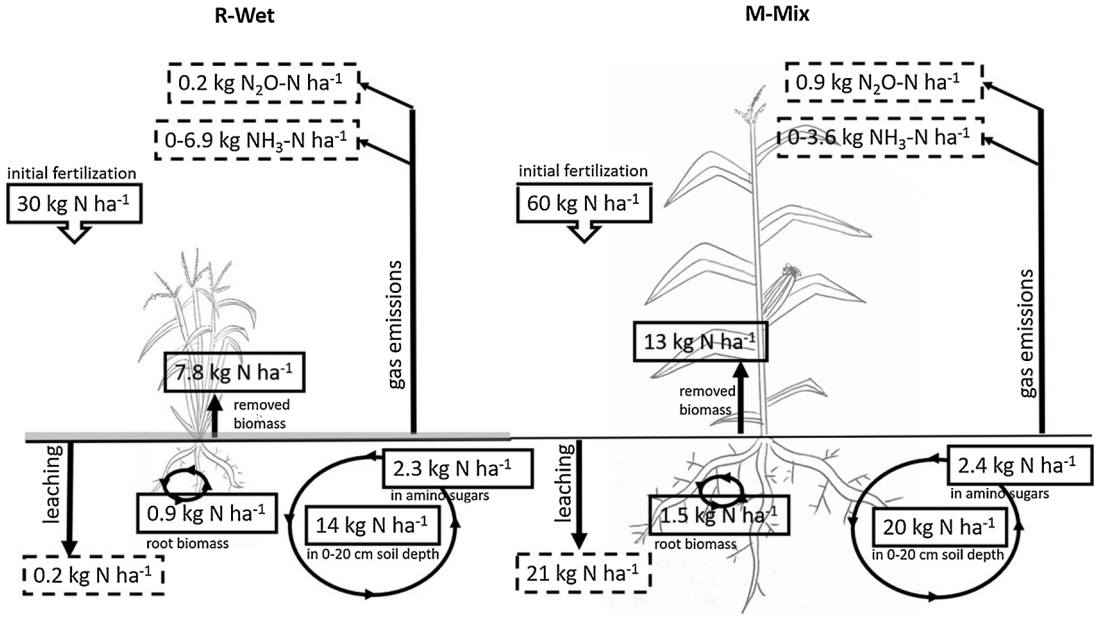

Fig. 5. Gross N fluxes for paddy rice – paddy rice (R-Wet) and maize – paddy rice (M-Mix) cropping systems during the first dry season after introducing maize into a continuous paddy rice cropping system. Residual-N (circled arrows) and N losses (leaching and gas emissions) refer to the first fertilization split (^{15}N urea) of the respective treatment. Values in boxes with dashed lines are estimated with the help of other studies: leaching losses are down-scaled based on He et al. (2017). N_2O-N emission losses were carefully up-scaled by referring the $^{15}N_2O$ losses to N_2O emission rates based on Weller et al. (2015). NH_3-N losses implies remaining N, which cannot be assigned to any N pool.

available anymore. This can be seen from low ^{15}N recoveries in the plant biomass compared to relatively high ^{15}N recoveries in the topsoil. Another abiotic immobilization process in clayey soils especially under anaerobic conditions of paddy systems is the immobilization of NH_4^+ in interlayers of clay minerals (e.g., Said-Pullicino et al., 2014). Ammonium fixation in interlayers of clay minerals might have been more intense for the R-Wet crop rotation than for the R-Dry and M-Mix rotations due to higher NH_4^+ concentrations in R-Wet and its slower oxidation (Ishii et al., 2011), especially during the dry season 2012.

4.3. N accumulation in soil microbial residues

The incorporation of N into microbial biomass can lead to an effective immobilization and retention of fertilizer N in soil. Amino sugar contents (as biomarkers for microbial residues) in our experiment were slightly larger than those reported for other paddy soils (Roth et al., 2011; Said-Pullicino et al., 2014) and non-paddy soils (Amelung et al., 2001b; Ding et al., 2011; Roth et al., 2011). The large clay content of more than 60% of the soils investigated at IRRI likely promoted the accumulation of amino sugars in soils because the fine soil particles protect microbial residues from decomposition by soil microorganisms (Said-Pullicino et al., 2014). Furthermore, the soil microbial activity in general might be enhanced with finer soil particles (Zhang et al., 1997).

The results show a correlation between amino sugar content and total N content, indicating a relevant contribution (> 7%) of amino sugar-N to N storage in soil. Although this correlation does not prove an efficient sequestration of fertilizer N in soil microbial residues, the relationship emphasizes the role of amino sugar for the soil N cycle and indicates biotic immobilization processes in all cropping systems.

In contrast to large absolute amino sugar-N proportions in M-Mix, only small recoveries of fertilizer ^{15}N in amino sugars in soil under M-Mix were observed. This is likely due to a dilution of ^{15}N enrichment by the large amino sugar background signal. This is a common phenomenon in isotope studies, which may be resolved by normalization to amino sugar content (Epron et al., 2011; Roth et al., 2011). The large content of Glu in soil under M-Mix diluted the isotope signal, whereas low amino sugar content of Gal and very low amino sugar contents of MurA did not contribute to this dilution. Yet, for indicating the total amount of ^{15}N immobilized in microbial residues, we plotted the average recovery of ^{15}N within the amino sugars. As Fig. 4 shows, up to 8% of total ^{15}N were recovered in the amino sugars. As the contribution of amino sugars to total cell wall N is small, with conversion factors of 9 for fungal glucosamine but at least of 45 for bacterial muramic acid (Apphuhn and Joergensen, 2006), more than 50% of total ^{15}N was found in microbial residues, thus more or less explaining the proportion of total ^{15}N recovered in soil (Fig. 2). We therefore have to conclude that microbial N immobilization is the major driver of ^{15}N retention in soil (Appuhn and Joergensen, 2006; Timsina and Connor, 2001). Soon after fertilization, a significant portion of this fertilizer N was still allocated to fungi, whereas particularly with prolonged cropping the bacterial impact on ^{15}N sequestration dominated. The ratio of Mur to Glu (APE MurA/Glu) in R-Wet for instance increased from 1.3 in the first dry season to 6.7 in R-Wet two years later (Fig. 4).

In experiments of Said-Pullicino et al. (2014), differences in fertilizer incorporation into microbial biomass between flooded and non-flooded soils were enhanced by straw incorporation, which worked as activator for microbial activity, like glucose in the experiments of He et al. (2011). Rice and maize stubbles (but no rice or maize straw) were incorporated into the soil in our experiment, which could have fostered microbial growth and reduced differences between concentrations of soil microbes and the composition of the soil microbial community under M-Mix and R-Wet. The stimulation of the soil microbial community might have promoted the immobilization of fertilizer ^{15}N first in microbial biomass and residues of all crop rotations.

4.4. Fertilizer N fate in N_2O after introducing upland crops

Although the δ^{15}N signature of emitted N_2O could only be estimated due to the extremely high level of ^{15}N labelling, our results correspond to the data from former studies pointing to the relevance of N_2O emissions and denitrification for the N balance of paddy systems (Cai 1996; Cai et al., 2002; Pathak et al., 2002; Weller et al., 2015; Zhao et al., 2009). Weller et al. (2015) measured higher N_2O emissions under M-Mix and R-Dry than under R-Wet. In our study involving the same experiment, initial N_2O emissions were also largest in R-Dry and M-Mix, followed by R-Wet during the dry season 2012. Weller et al. (2015)

stated that N_2O was further converted to N_2 in this system, due to larger water contents in the soil under dry rice compared to the soil under maize.

In our study, it is likely that N_2O was directly formed from fertilizer ^{15}N after its application as side-product of nitrification during the dry season, especially because it was applied in dissolved form. It is also possible that both nitrification and denitrification led to larger $\delta^{15}N$ values of emitted N_2O in the M-Mix and R-Dry crop rotations with strongly fluctuating soil water contents compared to the R-Wet rotation with constantly saturated soil water conditions. These findings underline the environmental influences of transforming continuous paddy rice fields to wet-dry crop rotations not only on the N cycle but also on greenhouse gas emissions.

4.5. Overall balance of the fertilizer N fate after introducing upland crops

When combining all data into one figure, backed up with findings from He et al. (2017) and Weller et al. (2015), we were able to draw a first picture of the fertilizer fate of the first labelled urea split application after introducing maize into a continuous paddy rice cropping system (Fig. 5). Although this being rather a conservative estimate, the direct comparison of M-Mix and R-Wet treatments shows that i) gross N fluxes in the M-Mix rotations are larger, due to larger initial fertilization amounts, ii) the share of ^{15}N taken up by the plants is more or less similar in both treatments, iii) N losses with drainage into groundwater (35%) and via N_2O emissions into the atmosphere are larger in M-Mix compared to R-Wet (both roughly 1%), leading to iii) a larger portion of ^{15}N remaining in soil for R-Wet (approx. 50%) compared to M-Mix (approx. 30%). Hence, introducing uplant crops into paddy fields does not only alter the N cycle within the system but also the effect of the cropping system on adjacent ecosystems. As a consequence, improving and ensuring the efficient fertilizer N usage of the maize crop is crucial for the success of maize – paddy rice cropping systems as keystone of the sustainable intensification of cereal production (FAO, 2016b).

5. Conclusion

The introduction of maize and dry rice reduced the recovery of fertilizer N in bulk soil and plants in rice cropping systems compared to continuous paddy systems directly after the introduction of maize and dry rice. A biotic immobilization of fertilizer N into the microbial biomass was observed in all cropping systems, initially also by fungi, but in the longer-term mainly by bacteria. Continuous paddy rice cropping increases the immobilization of fertilizer ^{15}N in microbial residues in comparison to maize – paddy rice and dry rice – paddy rice systems directly after the transformation, but differences between the cropping systems diminished within the following two years. Hence, the introduction of maize and upland crops into a continuous paddy rice cropping system reduces fertilizer N recovery, especially in the first year, most likely due to nitrate leaching and gaseous losses to the atmosphere.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.agee.2018.02.021.

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