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Article

Contribution of Biological Nitrogen Fixation and Ratoon Rice Growth to Paddy Soil Fertility: Analyses with Field Monitoring and Modeling Approach

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Abstract

At 6 rice paddy sites in 4 prefectures of Japan, we analyzed the contribution of biological N₂ fixation (BNF) and ratoon rice growth to soil N fertility, combining 2-year field monitoring and simulation by the biogeochemistry model DNDC-Rice. Across the sites and years, ratoon rice was found to accumulate up to 30 kg N ha⁻¹ without fertilization and irrigation after main rice harvest. Applying a newly built BNF model calibrated against literature data, BNF at the 6 sites were estimated to be 33–63 kg N ha⁻¹ yr⁻¹. Based on the simulations by DNDC-Rice under the locally typical managements, we estimated the contribution of BNF and ratoon rice to soil N fertility, being varied due to the climate, soil properties and management, as follows: (a) BNF and ratoon rice contributed 4–33% and 3–23% of N supply from soil during main rice season, respectively. (b) Contribution to main rice N uptake was 3–29% from BNF, whereas 6% or less from ratoon rice. (c) Although major part of N gain by BNF was being lost by denitrification and N leaching, BNF was contributing 88–349 kg N ha⁻¹ (1.5–6.6%) of the organic N pool in 0–30cm soil layer. Ratoon rice was working to save N loss by reducing N leaching, consequently contributing 14–183 kg N ha⁻¹ (0.2–3.3%) of the soil N pool. We suppose this is the first analysis that quantified the contribution of BNF and ratoon rice growth to paddy soil fertility.

Keywords: biological N₂ fixation; DNDC-Rice model; paddy rice; ratoon rice; soil fertility

1. Introduction

Paddy rice typically demands around 100 kg N ha⁻¹ crop⁻¹ or even more, but N fertilizer application on paddy rice is usually less than the expected N uptake. According to a study on N balance in Japanese farmlands[1], for example, average N fertilization to rice paddies in Tsukuba city in 1997 was 15% less than average N uptake by rice plant. Indigenous N supply from paddy soil is often referred to as “soil N fertility”. As N supply to rice directly influences its yield and quality, N fertilization should be deliberately designed weighing soil N fertility. Presumably, part of soil N fertility is attributed to N from previously applied crop residues and other organic matters such as composts and green manures. Another important N source that contributes to soil N fertility is biological N₂ fixation (BNF). According to a recent review[2], non-symbiotic BNF in rice, which was estimated by the means of 15N₂ feeding, 15N dilution or N balance, ranged 4–51 kg N ha⁻¹ crop⁻¹.

Another natural process that potentially influences paddy soil N fertility is growth of ratoon rice. In recent rice ratooning experiments conducted in southwestern Japan (33°12' N, 130°30' E), the total grain yield of main and ratoon rice reached 14.4 t ha⁻¹ in a year, when the main rice was harvested at the normal time leaving tall (50 cm) stubbles[3]. Even though economic or industrial production of ratoon rice is still limited in Japan, it is pretty common to see natural growth of ratoon rice, particularly in central Japan and south, when the field is left unplowed after harvest. Thus, it is credible that N uptake and C fixation by ratoon rice is making some contribution to N and C cycling in rice paddy soils.

If we want to quantify the contributions of BNF and ratoon rice to N and C cycling in rice paddy soils, an idealistic way is to experimentally eliminate BNF and ratoon rice from a rice paddy and compare the soils with/without BNF and ratoon rice. In reality, however, it will be difficult to completely inhibit these biological processes (BNF and ratoon rice growth) without causing any artifact, especially for a duration long enough (perhaps several years or even more) to observe significant changes in the N and C status of paddy soil. An alternative way to such real field experiments is simulation experiments that apply a numerical model to compute N and C cycling in rice paddies under hypothetical conditions with/without BNF and ratoon rice.

DNDC-Rice is a process-based biogeochemistry model developed especially for simulating N and C cycling in rice paddy ecosystems[4–11]. In this study, we added the DNDC-Rice model new components of BNF and ratoon rice growth which were calibrated with data from literature and field monitoring on rice paddies. Then, DNDC-Rice was applied to simulate the N and C cycling at the 6 monitoring sites for 2 years under hypothetical conditions with/without BNF and ratoon rice, in order to analyze what contributions BNF and ratoon rice make to the N fertility of rice paddy soils.

2. Materials and Methods

2.1. Monitoring Sites and Experimental Treatments

Table 1 gives a brief description of the 6 monitoring sites located in 4 prefectures of Japan. These 6 sites were chosen to represent, as much as possible, the regional variation of rice paddies in Japan as well as the typical paddy soil types in respective regions. The experiments in this project were designed primarily for testing reduction of chemical fertilizer usage with application of organic materials. The experimental treatments for the years 2023 and 2024 are summarized in Tables 2 and 3, respectively. Paddy rice was successively cultivated for the last 10 years or longer at each site except the SH5 site, where rice-rice-wheat-soybean (4 crops in 3 years) rotation was carried out for the last 21 years, and the crop cultivated in 2023 was rice after soybean. At each site, seedlings of locally major cultivars of Japonica rice were transplanted in spring and harvested in autumn. Each site, except the HO2 site, had both the CT (control) and OM (organic materials) plots. The CT plot followed the locally typical farming management for 10 years or longer, with or without the crop residues returned to the soil. In the OM plot, on the other hand, a kind of compost was applied to reduce the application rate of chemical fertilizer as compared to the CT plot. Additional plots were RN (reduced N fertilizer application without compost) and 0N (no N application) at the HO1 site, OMED (organic materials with extended midseason drainage) at the AO3 site, RS (rice straw applied instead of compost), 0N and OMDT (organic materials with deep tillage in every second year) at the NI4 site.

Depending on the sites, some of these treatments were started in 2023 or 2024. At the AO3 site in 2023, also, 2 cultivars (Masshigura and Harewatari) were cultivated under each treatment, and consequently the result of field soil incubation was identical for these 2 cultivars.

Table 1. Site location and soil properties.

Site	HO1	HO2	AO3	NI4	SH5	SH6
City, prefecture	Iwamizawa, Hokkaido	Iwamizawa, Hokkaido	Kuroishi, Aomori	Nagaoka, Niigata	Omihachiman, Shiga	Omihachiman, Shiga
North latitude, east longitude in degrees	43.18, 141.72	43.18, 141.72	40.67, 140.58	37.44, 138.87	35.18, 135.61	35.17, 136.13
Average air temperature (°C) for 1991-2020	7.9	7.9	10.2	13.3	14.5	14.5
Soil type (Comprehensive Soil Classification System of Japan First Approximation)[18]	Epi-mineralic Low-moor Peat soil	Fine-textured Epi-gray Gley Lowland soil	Epi-gray Gley Lowland soil	Fine-textured Mottled Gley Lowland soil	Fine-textured Gleyed Gray Lowland soil	Medium-textured Mottled Gley Lowland soil
Soil properties of the control plot ¹⁾						
Clay content (g g ⁻¹)	0.25	0.29	0.18	0.28	0.26	0.14
Bulk density (g cm ⁻³)	1.09	0.85	0.92	0.93	1.16	1.1
Carbon content (g g ⁻¹)	0.051	0.026	0.022	0.022	0.020	0.0155
pH	6.64	6.64	5.70	5.50	6.10	6.10
Reducible Fe content (mol kg ⁻¹)	0.103	0.103	0.103	0.134	0.160	0.1027
Water holding capacity (water filled pore space, cm cm ⁻³)	0.881	0.902	0.866	0.970	0.851	0.838

¹⁾ Data in Italics represent the average for the relevant Soil Series Group or Soil Group.

Table 2. Experimental treatments at each site in 2023.

Site	Plot	Main rice cultivar	Transplanting and harvest dates (MM/DD-MM/DD)	N Fertilization (kg N ha ⁻¹)	Organic materials application	Crop residues	Tillage date (MM/DD)	Drained period ¹⁾ (MM/DD-NM/DD)	Monitoring ²⁾
HO1	CT	Nanatshuboshi	5/16-9/4	65	-	-	4/28	ID 7/25-8/25	ABC
	OM			55	Rice straw compost (1.1 t C ha ⁻¹ , C/N 13.1, started in 2023)	-			AB
	RN			55	-	-			AB
	0N			0	-	-			AB
HO2	CT	Nanatshuboshi	5/30-9/5	65	-	-	5/9	MSD 7/20-7/26	C
AO3	CT1	Masshigura	5/25-10/14	80	-	Rice straw (2.0 t C ha ⁻¹ , C/N 60)	5/2	MSD 6/28-7/10	ABC
	CT2	Harewatari		80	-				ABC
	OM1	Masshigura		60	Chicken manure compost (0.6 t C ha ⁻¹ , C/N 7.2, started in 2023)				ABC
	OM2	Harewatari		60					ABC
NI4	CT	Koshihikari	5/10-9/9	60	-	-	4/17	MSD 6/9-6/22	ABC
	OM1			48	Rice straw compost (0.5 t C ha ⁻¹ , C/N 12.2, successively applied)	-	4/17	ID 6/22-8/25	ABC
	OM1DT			42	-	4/17	(deeper tillage every other year)	AB	

(deeper tillage every other year)

	RS			56	-	Rice straw (0.6 t C ha ⁻¹ , C/N 60)	4/17, 10/1		ABC
	0N			0	-	-	4/17		ABC
SH5	CT	Mizukagami, 3 crops (rice-rice-wheat-soybean) in 4 years	5/2-8/23	15	-	Rice straw (2.5 t C ha ⁻¹ , C/N 60) Wheat straw (1.2 t C ha ⁻¹ , C/N 97.2) Soy residue (1.0 t C ha ⁻¹ , C/N 54.6)	4/24, 10/29	MSD 6/12-6/26	ABC
	OM		5/2-8/21	0	Cattle manure compost (5.5 t C ha ⁻¹ , C/N 13.2, successively applied after rice harvest)	Wheat straw (2.2 t C ha ⁻¹ , C/N 91.3) Soy residue (1.2 t C ha ⁻¹ , C/N 53.9)			ABC

¹⁾ ID, intermittent drainage; MSD, midseason drainage

²⁾ A, main rice; B, ratoon rice; C, field soil incubation

Table 3. Experimental treatments at each site in 2024.

Site	Plot	Main rice cultivar	Transplanting and harvest dates (MM/DD-MM/DD)	N Fertilization (kg N ha ⁻¹)	Organic materials application	Crop residues application	Tillage date (MM/DD)	Drained period ¹⁾ (MM/DD-NM/DD)	Monitoring ²⁾
HO1	CT	Nanatshuboshi	5/17-9/2	65	-	-	4/25	ID 8/5-8/25	ABC
	OM			60	Rice straw compost (0.7 t C ha ⁻¹ , C/N 12.9)		-	ABC	
	RN			60	-	-	AB		
	0N			0	-	-	AB		
AO3	CT2	Harewatari	5/23-10/8	80	-	Rice straw (2.0 t C ha ⁻¹ , C/N 60)	5/3	MSD 6/26-7/5	ABC
	OM2			46	Swine manure		ABC		
	OM2ED			46	compost (0.5 t C ha ⁻¹ , C/N 7.7)		MSD 6/19-7/5	AB	
NI4	CT	Koshihikari	5/10-9/10	60	-	-	4/16	MSD 6/10-6/20	ABC
	OM1			48	Rice straw compost	-	ID 6/20-8/28	ABC	
	OM1DT			42	(0.5 t C ha ⁻¹ , C/N 12.2, successively applied)	-	4/16 (deep tillage every other year)	AB	
	RS			56	-	Rice straw (0.6 t C ha ⁻¹ , C/N 60)	4/16, 10/6	ABC	
	OM2			48	Chicken manure compost (0.4 t C ha ⁻¹ , C/N 10.3, started in 2024)	-	4/16	ABC	

	0N			0	-	-			ABC
SH6	CT	Koshihikari	5/13-8/27	60	-	Rice straw (2.5 t C	4/15, 11/13	MSD 6/10-6/17	ABC
	OM			50	Cattle manure	ha ⁻¹ , C/N 60)		MSD 6/10-6/24	ABC
					compost (1.0 t C ha ⁻¹ ,				
					C/N 12.7, started in				
					2024)				

¹⁾ID, intermittent drainage; MSD, midseason drainage

²⁾A, main rice; B, ratoon rice; C, field soil incubation

2.2. Field Soil Incubation for Measuring Soil N Supply

Soon after puddling or transplanting, wet soil was sampled from the plow layer (with a thickness of 13-15 cm), homogenized removing coarse organic matters such as fresh plant residues, then filled into cylindrical PE containers ($\phi 75$ mm $\times$ 91 mm). Each container filled with the wet soil was tightly closed with a screw lid, placed back into middle of the plow layer, and incubated during the rice growing season. Periodically, the PE containers were collected for analysis as follows: after removing 2-cm thick uppermost layer, the wet soil was homogenized and extracted with 1 M KCl solution to determine NH_4^+ content. Soil N supply was determined as the increase in soil NH_4^+ from the start of incubation. As fresh plant residues had been mostly removed prior to soil incubation, soil N supply measured this way presumably excluded N mineralization and/or immobilization caused by decomposition of fresh residues.

2.3. Measurements of Main Rice and Ratoon Rice Growth

At each site, as mentioned earlier, seedlings of locally major Japonica rice cultivar were transplanted and cultivated following locally typical management, except the experimental treatments of organic matter and fertilizer application. Periodically until maturity, stems, leaves and panicles (if present) were sampled and measured for dry weight, total C and N content.

As harvest of ratoon rice was not intended at any monitoring site, no fertilizers nor irrigation were applied after harvest of main rice. Thirteen to 72 days after harvest, depending on the sites, treatments and years, ratoon rice was sampled from 4-10 hills per replicate, and analyzed for total dry weight, total C and N content. Data acquired this way were analyzed to build and calibrate a ratoon rice growth model as described in the Appendix.

2.4. Meteorological Data Acquisition

To run the DNDC-Rice model as well as to analyze the main and ratoon rice growth, we acquired daily meteorological data (maximum and minimum air temperatures, precipitation, solar radiation, wind speed and relative humidity) at the latitude/longitude of each site from the Agro-Meteorological Grid Square Data, NARO (<https://amu.rd.naro.go.jp/>). As this system does not provide wind speed and relative humidity before 1988, wind speed of 2.0 m sec^{-1} and relative humidity of 70% were substituted for the lacking data, respectively.

2.5. Brief Description of the DNDC-Rice Model

DNDC-Rice simulates plant growth and soil processes at daily time step depending on input data on meteorology, soil properties, and farming managements. It was developed modifying the original DNDC model [12–14] especially on redox processes in rice paddy soils and production and emission of CH_4 .

In this study, further modifications and additions were made to DNDC-Rice in order to analyze the effects of BNF and ratoon rice growth on N dynamics in paddy soils under different conditions. BNF was assumed to be done by photosynthetic autotrophs on paddy surface, and the parameters in newly built BNF sub-model were calibrated according to literature data on BNF measured at a Japanese rice paddy site[15]. Another sub-model was built to simulate ratoon rice growth depending on the air temperature and soil N availability after main rice harvest and calibrated based on the site-specific monitoring data in this study. Decomposition (mineralization) of composts was simulated dividing them into 2 groups responding to their decomposing rate. Details of the construction, modification and calibration of DNDC-Rice are described in the Appendix.

2.6. Application of the DNDC-Rice Model

To analyze the contributions of BNF and ratoon rice growth to paddy soil N fertility in this study, DNDC-Rice was applied on each plot under following 3 settings: including BNF and ratoon rice growth (S1), excluding ratoon rice growth (S2), and excluding both BNF and ratoon rice growth (S3).

At all sites except the SH5 site, simulation was started from the year 2001. At the SH5 site, simulation was started from 2002 in order to repeat the 3-year cycle (rice-rice-wheat-soybean) 7 times until 2022. Up to 2022, farming managements (including fertilizer and compost application) were set the same as those in 2023, except for the plots where new treatment began in 2023 or 2024. Nitrogen input with precipitation and irrigation was estimated assuming N concentration of 0.5 ppm (0.25 ppm each for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) in precipitation and irrigation water.

Soil properties of the control plot (Table 1) were used as the initial values for all plots at each site. When measured soil data were not available, the average for relevant Soil Series Group or Soil Group was retrieved from the SolphyJ database [16] or a previous publication[17].

As mentioned before, most fresh plant residues were removed from the soil samples prior to the field incubation experiment. Such a specific condition was reproduced in the simulation by numerically setting all plant residues to zero, just on the beginning of field incubation in 2023 and 2024, only at the specific depth of soil (5cm) where soil N supply was compared between the observation and the simulation.

2.7. Statistical Analysis

On the results of field experiments, we tested the statistical significance of treatment effects on main rice growth, ratoon rice growth and soil N supply at respective sites. For this purpose, unpaired Student's *t*-test was performed between the relevant variables observed in the control plot and each treatment plot, applying the TTEST function of Microsoft Excel. Accuracy of model prediction was evaluated calculating the bias, correlation coefficient (*r*) and root mean square error (RMSE).

3. Results and Discussion on the Field Monitoring

3.1. Observed Rice Growth Under Various Conditions

Tables 4 and 5 summarize observed results of main rice growth and soil N supply in field incubation for 2023 and 2024, respectively. When replicate data were available, the result of *t*-test on the difference between the control and each treatment at each site is shown on the right-hand side of the S.D. (**, * and ns indicate $P < 0.01$, $P < 0.05$ and no significance, respectively).

TDD₁₀ (sum of daily mean air temperature over 10 °C) from transplanting to harvest was less than 1300 °C days at the northernmost site (HO1) but was close to or over 1600 °C days at the other sites.

At the HO1 site, reduction in N fertilizer application by 5-10 kg N ha⁻¹ (OM and RN plots) did not significantly affect grain yield nor N uptake, though complete elimination of N fertilizer (0N plot) apparently decreased grain yield and aboveground N uptake in 2023.

At the AO3 site, application of chicken manure compost in 2023 and swine manure compost in 2024 (coupled with reduction in N fertilizer application) did not significantly change grain yield in either cultivar (OM1 and OM2 plots), while the chicken manure compost significantly increased aboveground N uptake in the cultivar Harewatari (OM2 plot in 2023). On the other hand, 7-day longer midseason drainage in 2024 (OM2ED plot) significantly decreased grain yield.

At the NI4 site in 2023, successive application of rice straw compost or rice straw (OM1, OM1DT and RS plots) increased N uptake, though statistical significance was not found. Nonetheless, application of rice straw compost combined with biennial deeper tillage (OM1DT plot) significantly increased grain yield in 2023. In the plot without N application for more than 20 years (0N plot), grain yield was significantly reduced in 2023, but the rice plant absorbed more than 60 kg N ha⁻¹ in both years, indicating the potential of soil N fertility.

At the SH5 site in 2023, successive application of cattle manure compost (OM plot) significantly increased main rice N uptake, though observed increase in grain yield was not statistically significant. At the SH6 site in 2024, observed increase in grain yield and N uptake due to cattle manure compost (OM plot) was less than 7% as compared to the control.

Across all the sites, treatments and years, grain yield of rice was found to be highly correlated ($r = 0.847$, $n = 30$) to aboveground N uptake.

3.2. Soil N Supply Measured by Field Soil Incubation

The period of field soil incubation mostly matched the main rice growing period except at the HO1 and HO2 sites, where field soil incubation was terminated after 70 days because of technical reasons. Despite the shorter incubation period and relatively low air temperature, peat soil of the HO1-CT plot supplied 60-70 mg N kg⁻¹, which was nearly double that from the gley soil of nearby site (HO2-CT). Two-year application of rice straw compost to the HO1-OM plot did not significantly change soil N supply as compared to the control (HO1-CT plot).

Comparing the control plots of all sites, the soil of AO3-CT plot showed the highest N supply in both years. This is presumably because (a) this site received successive application of rice straw, and (b) the incubation period at this site was relatively long. It is notable that the first application of chicken manure compost in 2023 (AO3-OM plot) increased N supply by more than 50 mg N kg⁻¹ as compared to the control (AO3-CT plot). Considering the application rate of chicken manure compost (ca. 60 mg N kg⁻¹ soil), approximately 80% of the N in chicken manure compost was presumably mineralized during the incubation period. Also, the application of swine manure compost in 2024 (AO3-OM plot) significantly increased soil N supply.

No replicates were prepared in the field soil incubation experiment at the NI4 site. In the NI4-OM1 plot, nonetheless, successive application of rice straw compost increased soil N supply by 21-28 mg N kg⁻¹ as compared to the control. First application of chicken manure compost to the NI4-OM2 plot in 2024 increased soil N supply by 16 mg N kg⁻¹. It is noteworthy that soil of the NI4-0N plot, which received no N application for more than 20 years, supplied more than 60 mg N kg⁻¹ during the rice growing season of both years supporting growth of rice.

At the SH5 site in 2023, successive application of cattle manure compost (SH5-OM plot) nearly doubled observed soil N supply as compared to the control (SH5-CT plot). First application of cattle manure compost to the SH6-OM plot in 2024 significantly increased soil N supply by 11 mg N kg⁻¹ (21%) as compared to the control (SH6-CT plot).

Table 4. Observed results of main rice growth and field soil incubation in 2023.

Site-plot	Main rice growth									Field soil incubation				
	TDD ₁₀ from transplanting to harvest (°C days)	Grain yield (t ha ⁻¹)		Aboveground N (kg N ha ⁻¹)			Straw N (kg N ha ⁻¹)			Duration (days)	Mineralized N (mg N kg ⁻¹)			
		Ave.	(S.D.)	Ave.	(S.D.)	ns	Ave.	(S.D.)	ns					
											Ave.	(S.D.)		
HO1-CT	1280.2	5.11	-	92.5	(8.0)		34.1	(2.6)		70	70.7	(3.4)		
HO1-OM	1280.2	4.71	-	91.8	(8.7)	ns	34.5	(3.5)	ns	-	-	-		
HO1-RN	1280.2	5.03	-	85.5	(10.8)	ns	32.0	(4.2)	ns	-	-	-		
HO1-0N	1280.2	3.62	-	70.4	-	-	29.6	-	-	-	-	-		
HO2-CT	-			-	-		-	-		70	38.8	(1.1)		
AO3-CT1	1792.7	6.28	(0.52)	115.9	(18.1)		77.2	(13.0)		125	82.4	(2.7)		
AO3-CT2	1792.7	6.32	(0.62)	90.1	(19.2)		59.2	(13.9)						
AO3-OM1	1792.7	7.11	(0.27)	ns	141.2	(15.0)	ns	85.6	(8.5)	ns	125	135.1	(2.5)	**
AO3-OM2	1792.7	6.21	(0.06)	ns	143.1	(6.4)	*	82.2	(3.8)	ns				
NI4-CT	1895.2	5.43	(0.35)		88.1	(6.3)		28.6	(1.8)		120	73.3	-	
NI4-OM1	1895.2	5.62	(0.17)	ns	101.2	(4.6)	ns	31.5	(1.8)	ns	120	101.4	-	
NI4-OM1DT	1895.2	6.31	(0.26)	**	124.8	(40.0)	ns	39.9	(11.0)	ns	-	-	-	
NI4-RS	1895.2	5.92	(0.98)	ns	109.7	(17.7)	ns	33.2	(7.6)	ns	120	76.3	-	
NI4-0N	1895.2	3.80	(0.23)	**	62.7	(3.6)	ns	21.4	(2.4)	ns	120	62.8	-	
SH5-CT	1632.0	4.51	(0.83)		99.0	(6.6)		40.0	(7.0)		114	66.0	(1.0)	
SH5-OM	1591.6	5.97	(0.73)	ns	138.7	(7.7)	*	55.3	(5.3)	ns	114	128.8	(2.3)	**

Table 5. Observed results of main rice growth and field soil incubation in 2024.

Site-plot	Main rice growth										Field soil incubation			
	TDD ₁₀ from transplanting to harvest (°C days)	Grain yield			Aboveground N			Straw N			Duration (days)	Mineralized N		
		(t ha ⁻¹)			(kg N ha ⁻¹)			(kg N ha ⁻¹)				(mg N kg ⁻¹)		
		Ave.	(S.D.)		Ave.	(S.D.)		Ave.	(S.D.)			Ave.	(S.D.)	
HO1-CT	1171.4	4.55	(0.41)		82.2	(5.7)		23.6	(2.9)		70	58.2	(0.5)	
HO1-OM	1171.4	4.37	(0.38)	ns	79.7	(23.9)	ns	25.7	(6.5)	ns	70	57.9	(0.8)	ns
HO1-RN	1171.4	4.37	(0.28)	ns	77.1	(15.8)	ns	22.7	(6.5)	ns	-	-	-	
HO1-0N	1171.4	4.34	(0.18)	ns	76.2	(14.6)	ns	24.0	(4.0)	ns	-	-	-	
AO3-CT2	1625.8	6.55	(0.45)		115.4	(13.6)		38.2	(5.0)		98	70.8	(2.3)	
AO3-OM2	1625.8	6.40	(0.27)	ns	116.0	(3.5)	ns	39.6	(2.5)	ns	98	84.2	(2.5)	**
AO3-OM2ED	1625.8	5.37	(0.42)	*	90.9	(6.1)	ns	31.9	(3.3)	ns	-	-	-	
NI4-CT	1843.4	5.26	-		105.1	-		31.7	-		112	67.7	-	
NI4-OM1	1843.4	5.25	-		80.3	-		25.4	-		112	89.2	-	
NI4-OM1DT	1843.4	5.30	-		89.8	-		27.4	-		-	-	-	
NI4-RS	1843.4	5.05	-		98.1	-		30.6	-		112	70.0	-	
NI4-OM2	1843.4	5.51	-		109.3	-		30.2	-		112	83.6	-	
NI4-0N	1843.4	4.06	-		61.7	-		17.6	-		112	65.0	-	
SH6-CT	1629.2	5.02	-		93.2	-		33.0	-		106	55.6	(2.7)	
SH6-OM	1629.2	5.33	-		99.3	-		35.1	-		106	67.2	(2.0)	**

3.3. Ratoon Rice Growth Observed Under Various Conditions

Tables 6 and 7 summarize ratoon rice growth observed in 2023 and 2024, respectively. When replicate data were available, the result of *t*-test on the difference between the control and each treatment at each site is shown on the right-hand side of the S.D. (**, * and ns indicate $P < 0.01$, $P < 0.05$ and no significance, respectively).

TDD₁₀ for the ratoon rice growth period ranged 38.8-950.8 °C days among the sites for the 2 years. Across the sites, treatments and years, dry weight, N and C of ratoon rice were correlated to TDD₁₀ with $r = 0.814$ ($n = 29$), $r = 0.743$ ($n = 29$) and $r = 0.852$ ($n = 21$), respectively. The average of C/N ratio of ratoon rice measured at 3 sites was 30.3 g g⁻¹ and 26.1 g g⁻¹ in 2023 and 2024, respectively. As the result of *t*-test performed on available data, significant treatment effects on these variables were found only at the AO3 site.

Ratoon rice at the SH5 site in 2023 grew for the longest period and accumulated more than 30 kg N ha⁻¹. Ratoon rice dry weight observed at this site (2.1-2.5 t ha⁻¹) was comparable to that observed in the rice ratooning experiments in southwestern Japan when the main rice was harvested leaving short (20cm) stubbles [3].

Although ratoon rice at the AO3 site grew for the shortest period among the 4 sites in 2023, its dry weight and N were comparable to or higher than those observed at the HO1 site. Given the fact that N content in main rice straw at the AO3 site in 2023 (59-86 kg N ha⁻¹) was almost double that at the HO1 site (30-35 kg N ha⁻¹), above finding suggests that C and N remaining in the main rice stubbles enhanced the growth of ratoon rice, as reported in literature[19,20]. Recently, Nakano et al. [21] found that nonstructural carbohydrate content in the stubble regulates grain yield of ratoon rice in southwestern Japan.

Table 6. Observed ratoon rice growth in 2023.

Site-plot	Growth duration (days)	TDD ₁₀ (°C days)	Dry weight (t ha ⁻¹)			Ratoon N (kg N ha ⁻¹)			Ratoon C (kg C ha ⁻¹)		Ratoon C/N (g g ⁻¹)	
			Ave.	(S.D.)		Ave.	(S.D.)		Ave.	(S.D.)	Ave.	(S.D.)
HO1-CT	38	315.2	0.21	(0.06)		4.9	(1.1)		-	-	-	-
HO1-OM	38	315.2	0.23	(0.04)	ns	5.3	(0.8)	ns	-	-	-	-
HO1-RN	38	315.2	0.23	(0.04)	ns	5.2	(0.8)	ns	-	-	-	-
HO1-0N	38	315.2	0.31	-		6.5	-		-	-	-	-
AO3-CT1	13	38.8	0.40	(0.05)		5.9	(1.5)		200.4	(65.7)	33.9	(2.5)
AO3-CT2	13	38.8	0.46	(0.16)		4.9	(0.8)		171.6	(22.4)	35.0	(1.3)
AO3-OM1	13	38.8	0.61	(0.04)	*	9.4	(0.1)	ns	267.7	(8.5)	28.4	(0.6) ns
AO3-OM2	13	38.8	0.62	(0.02)	ns	9.6	(0.1)	ns	264.2	(16.5)	27.5	(1.4) *
NI4-CT	31	373.7	0.79	(0.20)		9.5	(2.9)		317.5	(80.4)	33.7	(1.8)
NI4-OM1	31	373.7	1.11	(0.07)	ns	14.7	(0.7)	ns	450.0	(28.9)	30.6	(0.6) ns
NI4-OM1DT	31	373.7	1.21	(0.01)	ns	17.1	(0.7)	ns	491.0	(1.1)	28.8	(1.1) ns
NI4-RS	-	-	-	-		-	-		-	-	-	-
NI4-0N	31	373.7	0.87	(0.42)	ns	10.9	(6.1)	ns	357.9	(177.8)	33.6	(2.6) ns
SH5-CT	70	910.4	2.46	(0.53)		37.6	(12.5)		990.9	(210.0)	26.3	(3.3)
SH5-OM	72	950.8	2.08	(0.40)	ns	34.9	(5.7)	ns	867.4	(173.5)	24.9	(0.9) ns
Mean											30.3	

Table 7. Observed ratoon rice growth in 2024.

Site-plot	Growth duration (days)	TDD ₁₀ (°C days)	Dry weight (t ha ⁻¹)			Ratoon N (kg N ha ⁻¹)			Ratoon C (kg C ha ⁻¹)			Ratoon C/N (g g ⁻¹)		
			Ave.	(S.D.)		Ave.	(S.D.)		Ave.	(S.D.)		Ave.	(S.D.)	
HO1-CT	18	175.3	0.19	(0.05)		5.16	(0.89)		-	-		-	-	
HO1-OM	18	175.3	0.18	(0.05)	ns	5.12	(1.06)	ns	-	-		-	-	
HO1-RN	18	175.3	0.21	(0.02)	ns	5.50	(0.27)	ns	-	-		-	-	
HO1-0N	18	175.3	0.17	(0.04)	ns	5.13	(0.97)	ns	-	-		-	-	
AO3-CT2	36	84.1	0.43	(0.02)		10.25	(0.24)		173.05	(6.35)		16.9	(0.2)	
AO3-OM2	36	84.1	0.71	(0.06)	ns	14.47	(1.05)	ns	282.52	(23.91)	ns	19.5	(0.2)	**
AO3-OM2ED	36	84.1	0.68	(0.05)	ns	10.97	(0.93)	ns	276.49	(21.00)	ns	25.2	(0.2)	**
NI4-CT	24	359.7	0.59	(0.18)		9.19	-		245.13	-		26.7	-	
NI4-OM1	24	359.7	0.57	(0.07)	ns	9.87	-		234.67	-		23.8	-	
NI4-OM1DT	24	359.7	0.68	(0.11)	ns	10.98	-		280.12	-		25.5	-	
NI4-RS	24	359.7	0.46	-		8.63	-		192.94	-		22.4	-	
NI4-OM2	24	359.7	0.51	-		7.92	-		209.80	-		26.5	-	
NI4-0N	24	359.7	0.39	(0.11)	ns	6.30	-		162.33	-		25.8	-	
SH6-CT	66	946.9	1.75	-		18.93	-		717.24	-		37.9	-	
SH6-OM	66	946.9	1.47	-		16.52	-		605.69	-		36.7	-	
Mean												26.1		

4. Analyses Applying the DNDC-Rice Model

4.1. Simulated Biological N₂ Fixation

As shown in Tables 8 and 9, simulated BNF in 2023 and 2024 ranged 33-63 kg N ha⁻¹ yr⁻¹, depending mostly on the irradiance at paddy surface for flooded period ($r > 0.99$). Simulated BNF in the NI4-0N plot (63 kg N ha⁻¹ yr⁻¹) was higher than published values of seasonal N fixation observed in Japanese paddy soils, e. g. 45 kg N ha⁻¹ [15] and 35.5 kg N ha⁻¹[22], and similar to observed N uptake (63 kg N ha⁻¹) by the main rice in this plot without N fertilization. Other possible N sources, namely atmospheric deposition and irrigation water, were not monitored in this study. According to an analysis of long-term N deposition at 7 locations in Japan[23], nevertheless, average N deposition for the period of 2003-2018 was 11.5 kg N ha⁻¹ yr⁻¹. As for N input with irrigation water to rice paddies, a likely estimate is 15 kg N ha⁻¹ yr⁻¹[24]. Comparing with these estimates of different N sources, BNF is suggested to be a major N source for the NI4-0N plot.

Applying metagenomic analyses, Masuda et al. [25–27] recently found that Nitrogenase genes (*nif*) in paddy soil samples were derived predominantly from diazotrophic Fe-reducing bacteria such as *Anaeromyxobacter* and *Geobacter*, and proposed that these bacteria primarily drive N₂ fixation in paddy soils. It deserves attention that their findings do not agree previous ones such as (a) N₂ fixation in a paddy soil was accompanied by CO₂ fixation (C/N = ca. 6.0) indicating that N₂ fixers were autotrophs [15] and (b) N₂ fixation in a paddy soil was almost completely inhibited by shutting out the light, indicating that N₂ fixers were photosynthetic microorganisms[28]. It is difficult to conclude here what microorganisms are the primary N₂ fixers in paddy soils. As mentioned earlier, this study constructed the BNF model assuming photosynthetic autotrophs as the N₂ fixers. However, as the DNDC-Rice model includes an algorithm to explicitly calculate soil Fe reduction[4], it can be modified to couple soil Fe reduction and N₂ fixation when necessary.

Table 8. Simulated biological N₂ fixation in 2023.

Site-plot	Flooded period	Irradiance at paddy surface for flooded period	BNF
	(days)	(MJ m ⁻²)	(kg N ha ⁻¹ yr ⁻¹)
HO1-CT	93	777.5	44.2
HO1-OM	93	773.3	43.9
HO1-RN	93	782.9	44.5
HO1-0N	93	845.0	48.8
HO2-CT	76	691.4	38.0
AO3-CT1	103	888.9	51.0
AO3-OM1	103	886.1	50.8
NI4-CT	82	963.5	57.7
NI4-OM1	82	945.3	56.5

NI4-OM1DT	82	962.1	57.6
NI4-RS	82	958.6	57.4
NI4-0N	82	1042.3	63.1
SH5-CT	101	715.9	41.6
SH5-OM	101	699.3	40.5

Table 9. Simulated biological N₂ fixation in 2024.

Site-plot	Flooded period	Irradiance at paddy surface for flooded period	BNF
	(days)	(MJ m ⁻²)	(kg N ha ⁻¹ yr ⁻¹)
HO1-CT	96	789.5	44.9
HO1-OM	96	782.4	44.4
HO1-RN	96	790.7	45.0
HO1-0N	96	852.9	49.2
AO3-CT2	95	781.0	44.1
AO3-OM2	95	780.4	44.1
AO3-OM2ED	88	733.6	41.0
NI4-CT	88	973.4	58.7
NI4-OM1	88	952.6	57.2
NI4-OM1DT	88	967.0	58.3
NI4-RS	88	965.0	58.3
NI4-OM2	88	966.8	58.3
NI4-0N	88	1033.0	62.6
SH6-CT	101	646.0	34.2
SH6-OM	94	630.5	33.2

4.2. Simulation of Soil N Supply

Figure 1 compares soil N supply measured in the field incubation and simulated by the DNDC-Rice model for 2023 and 2024 under the 3 settings. As done earlier, the result of *t*-test on observed difference between the treatments and control is shown above the bar (**, * and ns indicate $P < 0.01$, $P < 0.05$ and no significance, respectively).

In the simulation with BNF and ratoon rice growth (S1), simulated soil N supply showed a high correlation with the observation ($r = 0.85$), though holding a negative bias ($-14.2 \text{ mg N kg}^{-1}$) with the RMSE of $21.1 \text{ mg N kg}^{-1}$. The northernmost sites (HO1 and HO2) were large error sources: soil N supply at these sites was underestimated by $22\text{--}55 \text{ mg N kg}^{-1}$. Excluding these 2 sites, the correlation between the simulated (S1) and observed soil N supply was raised to $r = 0.91$, and the bias was narrowed to $-8.2 \text{ mg N kg}^{-1}$, with the RMSE of $12.6 \text{ mg N kg}^{-1}$.

Depending on the conditions at each experiment site, different organic materials were applied for different number of years: rice straw compost for 2 years (HO1 site), chicken manure compost and swine manure compost for 1 year each (AO3 site), rice straw and rice straw compost for successive years and chicken manure compost for 1 year (NI4 site), cattle manure compost for successive years (SH5 site), cattle manure compost for 1 year (SH6 site). Figure 2 compares observed and simulated N supply due to organic material application, quantified as the difference between the OM/RS and CT plots. Due to the above-mentioned variations in experimental conditions, observed N supply from organic material application ranged $0\text{--}63 \text{ mg N kg}^{-1}$, and DNDC-Rice well predicted them except for the chicken manure compost applied at the AO3 site in 2023: excluding this sample, the RMSE was lowered from 11.4 ($n = 10$) to 6.3 ($n = 9$) mg N kg^{-1} .

4.3. Contribution of BNF and Ratoon Rice to Soil N Supply

In the simulation excluding ratoon rice growth (S2), soil N supply decreased (as compared to S1) by 8.3 and 9.4 mg N kg^{-1} as the average for 2023 and 2024, respectively. By excluding both BNF and ratoon rice growth (S3), it further decreased by 16.0 and $15.2 \text{ mg N kg}^{-1}$ for 2023 and 2024, respectively. These results suggest that BNF and ratoon rice are making substantial contributions to N supply from rice paddy soils.

Contribution of BNF to soil N supply, quantified as the difference between S2 and S3, was in the range $2\text{--}29 \text{ mg N kg}^{-1}$ across the sites, plots and years. It was high ($19\text{--}29 \text{ mg N kg}^{-1}$) at the NI4 site, reflecting the high BNF at this site (Tables 8 and 9). On the other hand, contribution of BNF at the SH5 site was the lowest level ($2\text{--}3 \text{ mg N kg}^{-1}$), presumably because (a) BNF occurred only in 2 rice seasons out of the 3-year rotation cycle, and (b) relatively high air temperature during non-rice season (autumn, winter and spring) at this site enhanced N mineralization outside the rice season. As compared to soil N supply observed at the control plots, relative contribution of BNF to soil N supply was estimated as $9\text{--}33\%$ at the single rice cropping sites, and 4% at the paddy-upland rotation site (SH5).

Contribution of ratoon rice to soil N supply, quantified as the difference between S1 and S2, was in the range $1\text{--}20 \text{ mg N kg}^{-1}$. It was relatively small at the northern sites (HO1, HO2 and AO3) where growth of ratoon rice was limited due to low air temperature after main rice harvest. At the southern sites (SH5 and SH6), on the other hand, ratoon biomass was larger than that at the NI4 site, but its contribution to soil N supply was smaller than that at the NI4 site. Similarly to the case of BNF, plausible reasons are (a) ratoon rice grew only after 2 rice seasons out of the 3-year rotation cycle, and (b) at the SH5 and SH6 sites, ratoon rice was plowed into the soil in October or early November, and relatively high temperature during non-rice season (autumn, winter and spring) at these sites enhanced N mineralization outside the rice season. As compared to soil N supply observed at the control plots, relative contribution of ratoon rice to soil N supply was estimated as $3\text{--}23\%$ at the 6 sites.

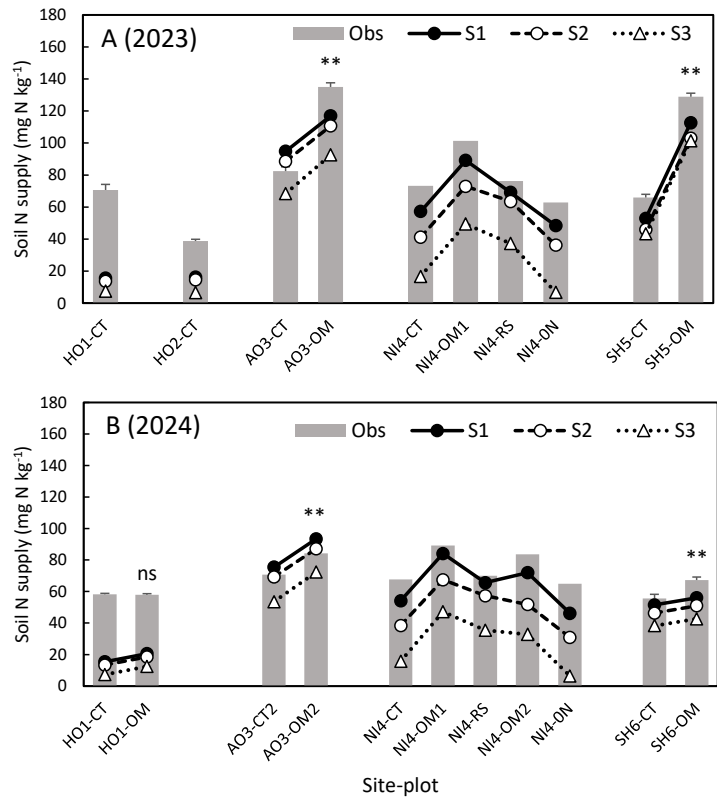


Figure 1. Observed and simulated soil N supply (mineralization) during the field soil incubation experiments in 2023 (A) and 2024 (B). S1, simulation with BNF and ratoon rice growth; S2, simulation without ratoon rice growth; S3, simulation without BNF and ratoon rice growth. As in Tables 4 and 5, **, * and ns indicate $P < 0.01$, $P < 0.05$ and no significance, respectively, for observed difference between the treatment and control.

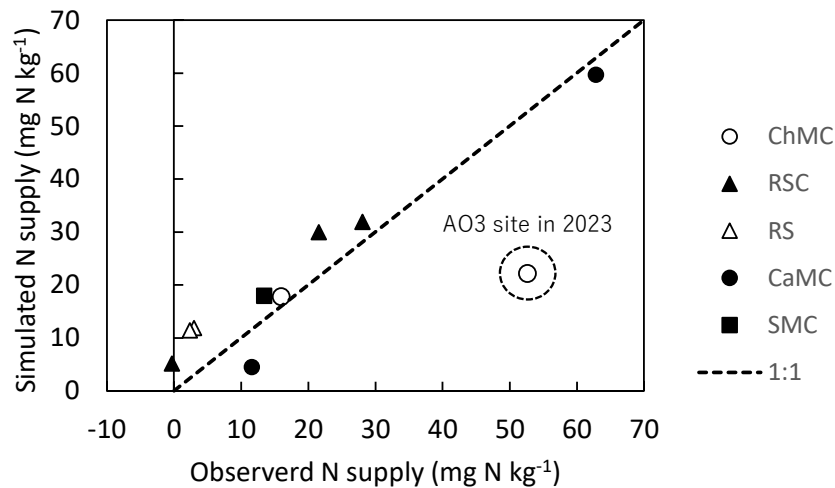


Figure 2. Comparison of observed and simulated soil N supply (mineralization) due to application of organic materials during the field soil incubation. ChMC, chicken manure compost; RSC, rice straw compost; RS, rice straw; CaMC, cattle manure compost; SMC, swine manure compost.

4.4. Contribution of BNF and Ratoon Rice to Main Rice N Uptake

Figure 3 compares main rice N uptake in 2023 and 2024 observed and simulated by DNDC-Rice under the 3 settings. In this study, we employed a relatively simple rice growth model based on effective temperature sum, using the same parameter set for all cultivars, except the TDD_{10max} for the cultivar Nanatsuboshi. Nevertheless, the simulation including BNF and ratoon rice growth (S1) reasonably reproduced the variation in N uptake ($r = 0.70$, RMSE = 16.0 kg N ha⁻¹, bias = 3.1 kg N ha⁻¹).

Quantified as the difference between S2 and S3, BNF contributed 3-49 kg N ha⁻¹ of main rice N uptake, reflecting the contribution of BNF to soil N supply (Fig. 1). In the NI4-0N plot, where no N was applied for more than 20 years, BNF accounted for 49 kg N ha⁻¹ of N uptake in both years. At the paddy-upland rotation site (SH5), on the other hand, contribution of BNF to N uptake was only 3-4 kg N ha⁻¹. As compared to the observed N uptake in the control plots at single rice cropping sites, BNF contributed 9-29% of main rice N uptake, similarly to the contribution to soil N supply. When compared to the N gain by BNF in the control plots (34-59 kg N ha⁻¹), on the other hand, N allocated to main rice was less than 50% of the N gain by BNF.

Quantified as the difference between S1 and S2, contribution of ratoon rice to main rice N uptake was 0-5 kg N ha⁻¹ across all sites, plots and years. As compared to the observed N uptake in the control plots, the relative contribution of ratoon rice was 0-6%, which is lower than its contribution to soil N supply (3-23%). This can be attributed to the relatively high C/N ratio (ca. 30) of ratoon rice. The N and C cycling of ratoon rice (N uptake, CO₂ fixation and production of organic N) worked to raise organic N content of the soil. However, when ratoon rice was plowed into soil prior to the next main rice season, decomposition of ratoon biomass induced N immobilization, competing with N uptake by main rice.

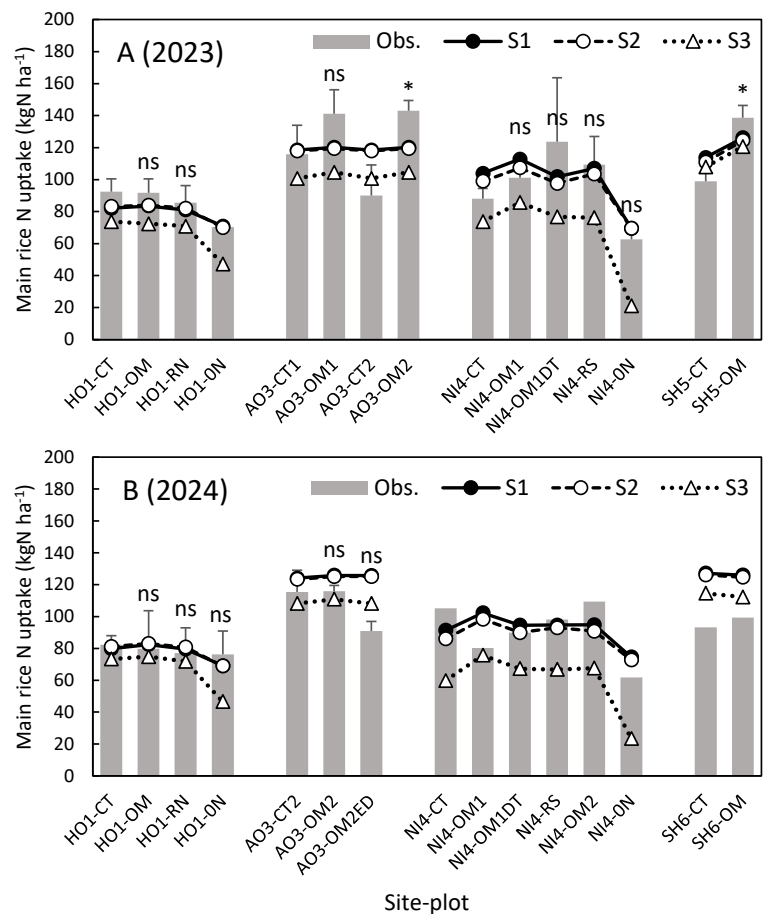


Figure 3. Observed and simulated main rice N uptake (aboveground N) in 2023 (A) and 2024 (B). S1, simulation with BNF and ratoon rice growth; S2, simulation without ratoon rice growth; S3, simulation without BNF and ratoon rice growth. As in Tables 4 and 5, **, * and ns indicate $P < 0.01$, $P < 0.05$ and no significance, respectively, for observed difference between the treatment and control.

4.5. Effects of BNF and Ratoon Rice on N Loss from Rice Paddies

Next, we analyzed how BNF and ratoon rice influence N loss from rice paddies. Figure 4 shows N loss due to denitrification and N leaching from the 0-50cm soil profile of control plot at each site simulated under the 3 settings (S1, S2 and S3).

Quantified as the difference between S2 and S3, BNF increased N loss by 20-45 kg N ha⁻¹ yr⁻¹, indicating that major part of the N gain by BNF was lost due to denitrification and leaching. At the HO1 and HO2 sites, BNF increased N loss mainly due to denitrification, because relatively high soil pH (6.64) at these sites favored denitrification (N₂ emissions accounted for most of the denitrification). When the input of soil pH was lowered to 5.6 on trial, simulated denitrification from the HO1-CT plot in 2024 was reduced to 2.2 kg N ha⁻¹ yr⁻¹, increasing N leaching by ca. 20 kg N ha⁻¹ yr⁻¹ in turn. In the AO3-CT and NI4-CT plots with soil pH of 5.7 and 5.5, respectively, BNF increased N loss mostly due to N leaching.

Ratoon rice gains no N, but it worked to save N loss. At the NI4, SH5 and SH6 sites, where ratoon rice grew relatively large, it reduced N leaching and saved N loss by 7-11 kg N ha⁻¹ yr⁻¹, quantified as the difference between S1 and S2. As there was no difference in N input between the S1 and S2 simulations, these results suggest that ratoon rice is working to raise soil N pool by saving N loss due to leaching.

4.6. Contribution of BNF and Ratoon Rice to Soil N Pool

Table 10 shows organic N pool in 0-30cm soil layer of each plot simulated with BNF and ratoon rice (S1), with BNF (S2), and without BNF and ratoon rice (S3). In Table 10, we left out some plots where new treatments began in 2023 or 2024 (namely HO1-OM, HO1-RN, HO1-0N, AO3-OM, NI4-OM2, and SH6-OM), because these plots had been identical to respective CT plots until 2023 or 2024, and consequently had virtually the same N pool as respective CT plots. The soil N pool of the SH6-CT plot is as of the end of 2024, while the others are as of the end of 2023.

Quantified as the difference between S2 and S3, contribution of BNF to the organic N pool under the locally typical managements (CT plots) was 88-349 kg N ha⁻¹, and equivalent to 1.5-6.6% of that pool. Although major part of the N gain by BNF was lost due to denitrification and N leaching (Fig. 4), it was still contributing to N pool of the paddy soils. The contribution of BNF was relatively small at the southern sites (SH5 and SH6), presumably because of the paddy-upland rotation (SH5 site) and/or the warmer climate that enhanced soil N mineralization throughout the year. With respect to treatment effects within respective sites, relative contribution of BNF was lower in the plots successively receiving organic matter application (NI4-OM1, NI4-OM1DT, NI4-RS, SH5-OM), and higher in the plot receiving no N application (NI4-0N).

Quantified as the difference between S1 and S2, ratoon rice was contributing 14-183 kg N ha⁻¹ (0.2-3.3%) to the soil organic N pool under the locally typical managements. While ratoon rice gained no N, it did contribute to building the organic N pool through uptake and immobilization of inorganic N. The contribution of ratoon rice was relatively small at the northernmost sites (HO1 and HO2) where growth of ratoon rice was limited due to the lower air temperature. Under the locally typical managements, combined contribution of BNF and ratoon rice to the soil organic N pool was 2.0-9.9%. Excluding the peat soil at the HO1 site, which had indigenously high C and N contents, the combined contribution of BNF and ratoon rice was 4.5-9.9%.

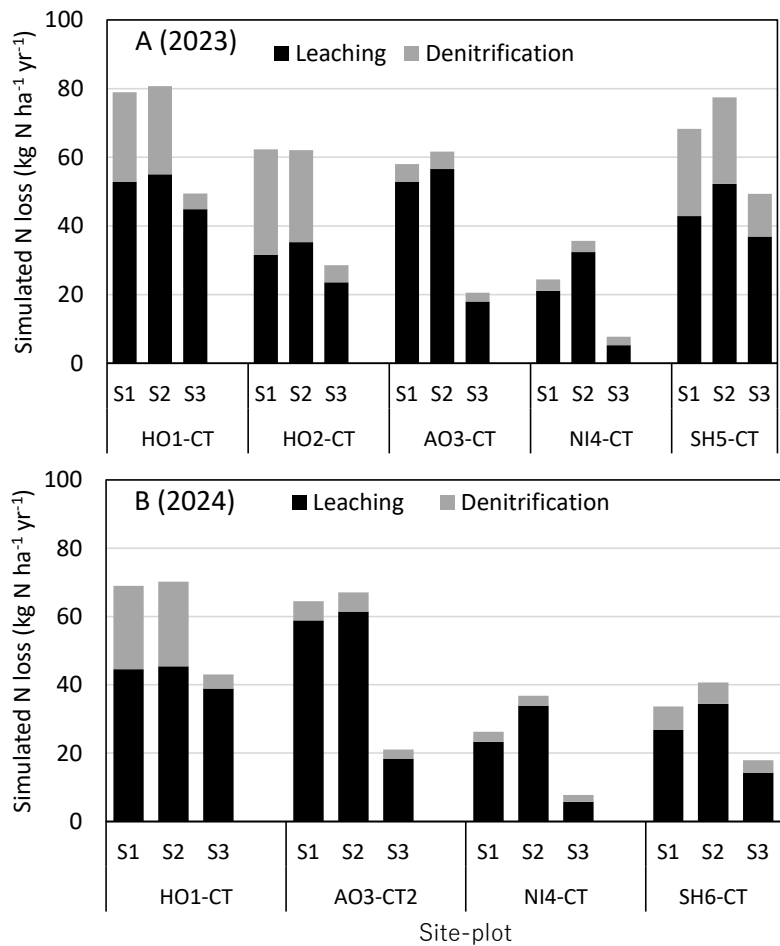


Figure 4. Simulated N loss (denitrification and N leaching) from the 0-50cm soil profile of control plot at each site in 2023 (A) and 2024 (B).

Table 10. Contributions of BNF and ratoon rice to soil N pool: Organic N in 0-30cm soil layer at the end of 2023 or 2024 simulated with BNF and ratoon rice (S1), with BNF (S2), and without BNF and ratoon rice (S3).

Site	HO1	HO2	AO3	NI4					SH5-CT	SH6	
Plot	CT	CT	CT	CT	OM1	OM1DT	RS	0N	CT	OM	CT
Simulated organic N in 0-30cm soil layer (kg N ha ⁻¹)											
S1	11716	5227	5772	5303	5726	5716	5383	5142	6016	7335	5212
S2	11699	5213	5695	5127	5514	5533	5289	4975	5833	7103	5104
S3	11478	4973	5356	4777	5205	5179	4974	4589	5745	7042	4931
Contribution to the organic N pool (kg N ha ⁻¹)											
BNF ¹⁾	221	240	339	349	309	354	316	386	88	61	172
Ratoon rice ²⁾	17	14	77	177	212	183	94	166	183	233	108
Sum	238	253	416	526	521	536	410	553	271	293	280
Contribution to the organic N pool (%)											
BNF	1.89	4.59	5.87	6.58	5.39	6.19	5.86	7.51	1.46	0.82	3.31
Ratoon rice	0.15	0.26	1.33	3.34	3.71	3.19	1.74	3.24	3.05	3.17	2.07

Sum	2.03	4.85	7.20	9.92	9.10	9.38	7.61	10.75	4.51	3.99	5.38
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- ¹⁾ Quantified as the difference between S2 and S3.
²⁾ Quantified as the difference between S1 and S2.

5. Conclusions

Through combining field monitoring and model simulations, we analyzed the contributions of biological N₂ fixation (BNF) and ratoon rice growth to soil N fertility at 6 rice paddy sites in Japan. For this purpose, the DNDC-Rice model was equipped with newly constructed sub-models of BNF and ratoon rice growth, which were calibrated against literature data or the site-specific monitoring data.

Applying the BNF model calibrated against the literature data, BNF at the 6 sites for the 2 years were estimated to be 33-63 kg N ha⁻¹ yr⁻¹. In the 2-year monitoring, ratoon rice accumulated up to 30 kg N ha⁻¹ without fertilization and irrigation, depending mainly on the air temperature after the harvest of main rice.

According to the simulations under locally typical managements, BNF and ratoon rice contributed 4-33% and 3-23% of N supply from the soil, respectively, during the main rice season. With respect to N uptake in the main rice, BNF contributed 3-29% of it, but the contribution of ratoon rice was 6% or less, presumably because of N immobilization induced by decomposition of ratoon rice of previous season. Contribution of BNF to soil N supply as well as main rice N uptake was the lowest at the paddy-upland rotation site.

Under the locally typical managements, BNF was contributing 0.8-7.5% of the organic N pool in 0-30cm soil layer. Ratoon rice was working to save N loss by reducing N leaching, contributing 0.2-3.3% of the soil organic N pool. Combined together, the contribution of BNF and ratoon rice to soil organic N pool was estimated to be 238 kg N ha⁻¹ (2.0%) for a peat soil, and 253-526 kg N ha⁻¹ (4.5-9.9%) for the other soils.

Above analyses are inevitably influenced by the uncertainties in simulations. The ratoon rice growth model was calibrated against the site-specific monitoring data, but the BNF model was calibrated against one set of literature data only. Therefore, the BNF model may need further calibration and validation to assure these results, although the agreement between measured and simulated soil N supply supports the relevance of simulated N₂ fixation.

The analyses in this study were limited to the 6 sites in 4 prefectures, while there are 47 prefectures in Japan, and over 200 soil series groups are recognized under its rice paddies according to the SolphyJ database [16]. Thus, we presented the results of case studies that do not represent the statistical average or range over the total rice paddies in Japan. For analyses of wider scale, further works will be required to improve and validate the models' applicability.

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Data Availability Statement: Full data from the field monitoring in 2023 and 2024 (main rice and ratoon rice growth as well as soil N supply during the field incubation) are available as the Supplementary Material.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BNF Biological Nitrogen (N₂) Fixation

Appendix A. Construction, Modification and Calibration of the DNDC-Rice Model

Appendix A.1. Modeling of SOC decomposition

Similarly to the original DNDC model, DNDC-Rice divides soil organic matters into 4 pools with specific C/N ratios and decomposition rates (i.e. plant residues, microbial biomass, humads, and passive humus), including labile and resistant components within the pools (Fig. S1). This scheme follows the NCSOIL model[29], and the individual parameters were partly revised or added in this study and earlier works[4]. As shown in Fig. S1, decomposed organic C is assumed to turn into CO₂, dissolved organic C (DOC) or next organic C pools at a fixed ratio. Depending on the N balance between the previous and new C pools, net N mineralization or immobilization occurs. Table S1 shows the C/N ratio and the specific decomposition rate (SDR) of each organic C pool, and Table S2 summarizes the functions to simulate organic C decomposition. As shown in Fig. S2, the SDR of labile and resistant residues were calibrated to reproduce published data of rice straw decomposition monitored at 2 paddy sites in central Japan[30,31].

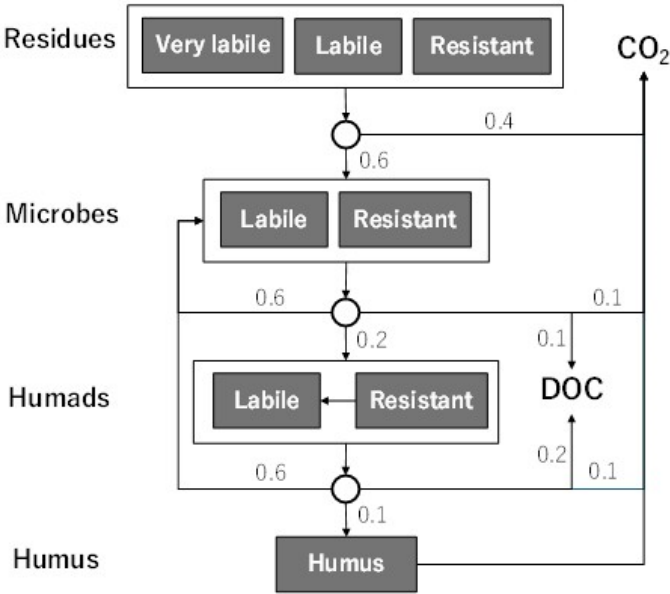


Figure A1. Schematic diagram of decomposition of soil organic matters in DNDC-Rice. Figures in the diagram indicate the ratio to partition decomposed organic C.

Table A1. C/N ratio and the specific decomposition rate (SDR) of the soil C pools in DNDC-Rice.

Pool		C/N	SDR (day ⁻¹)
Residues	Very labile	5	0.375
	Labile	60	0.105
	Resistant	200	0.00675
Microbial biomass	Labile	6	0.594
	Resistant	6	0.072
Humads	Labile	6	0.288

	Resistant	6	<i>0.0108</i>
Humus		11.3	<i>0.000274</i>

SDR values in italics were adjusted in this study.

Table A2. Functions to simulate organic C decomposition.

Function	Equation	Reference
Decomposition of C pools	$\frac{d}{dt}[C] = -SDR[C]f_Tf_Mf_Nf_{O_2}f_{clay}f_{till}$	
	f_T = effect of soil temperature	[4]
	f_M = effect of soil moisture	
	f_N = effect of N deficiency	
	f_{O_2} = effect of O ₂ concentration	
	f_{till} = effect of tillage	
	$f_{clay} = 1$ (for residues)	[32]
	$f_{clay} = 1.17 - 0.2 \ln(clay)$	
	$0.5 \leq f_{clay} \leq 1$	
	$clay$ = soil clay content (%)	

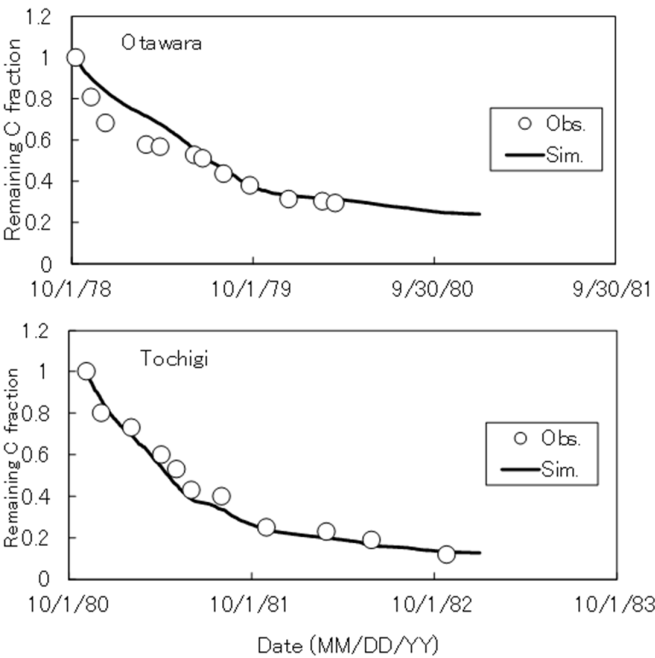


Figure A2. Observed and simulated rice straw decomposition at 2 paddy sites in Japan.

Appendix A.2. Modeling of Compost Decomposition

Considering the mineralization of different types of composts applied in the field experiments, we divided composts into 2 groups: slower decomposing ones (cattle manure and rice straw composts) and faster decomposing ones (chicken manure and swine manure composts), then constructed different functions to estimate their organic C compositions (Table A3).

As shown in Fig. A3, organic C composition of cattle manure and rice straw composts was estimated from the C/N ratio, based on published data of chemical analyses of cattle manure composts [33] and rice straw composts[34]. From these analyses, we derived the total fiber content (mainly hemicellulose, cellulose and lignin) as well as the hemicellulose and cellulose content in the compost samples. Comparing with the organic C pools in the DNDC-Rice model, we recognized hemicellulose and cellulose as the labile residue, and lignin as the resistant residue. The non-fibrous fraction of compost was regarded as the microbial biomass and humads.

The labile residue fraction in compost was approximated by a logarithmic function fitted to the measured hemicellulose and cellulose fraction (Fig. A3). Then, remaining C in the compost was partitioned into the resistant residue (C/N = 200) and the non-fibrous fractions (C/N = 6) according to the compost's C/N ratio. As no measured data were available to further partition the non-fibrous fraction, it was provisionally assumed to consist of 50% microbial biomass and 50% humads.

As shown in Fig. A4, the SDRs of humads and microbial biomass were adjusted by a single factor (keeping the original ratios between them) to reproduce the 5-year (1976-1981) decomposition data of 3 samples of rice straw compost with different C/N ratios published in a governmental report [35]. The RMSE of remaining C fraction after 5 years was 0.016 g g⁻¹.

To reproduce the fast mineralization of chicken manure and swine manure composts, on the other hand, they were provisionally assumed to consist of very labile (C/N = 5) and labile (C/N = 60) residues, and partitioned into these 2 fractions according to their C/N ratio.

Table A3. Functions to partition compost into organic C fractions.

Variable	Equation
Compost C/N ratio	$6 \leq x \leq 40$
Cattle manure or straw compost	
Labile residue fraction	$LR = 0.155 \ln(x - 9)$
Non-fibrous fraction	$OCC = \frac{r_0 - r_2 - LR(r_3 - r_2)}{r_1 - r_2}$ $r_0 = \text{N/C ratio of compost (1/x)}$ $r_1 = \text{N/C ratio of non-fibrous fraction (1/6)}$ $r_2 = \text{N/C ratio of resistant residue fraction (1/200)}$ $r_3 = \text{N/C ratio of labile residue fraction (1/60)}$
Resistant residue fraction	$RR = 1 - LR - OCC$
Humads and microbial biomass fractions	$HMD = MBC = 0.5 OCC$
Chicken or swine manure compost	
Labile residue fraction	$LR = \frac{r_0 - r_4}{r_3 - r_4}$ $r_0 = \text{N/C ratio of compost (1/x)}$ $r_3 = \text{N/C ratio of labile residue fraction (1/60)}$ $r_4 = \text{N/C ratio of very labile residue fraction (1/5)}$
Vary labile residue fraction	$VLR = 1 - LR$

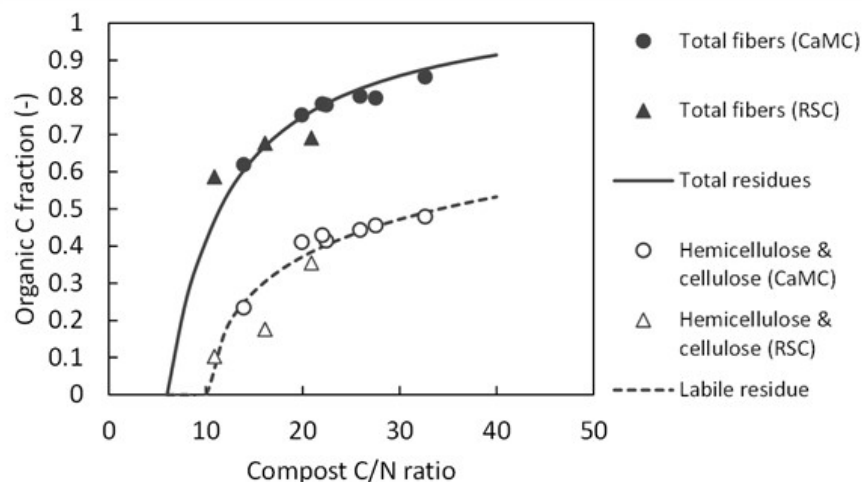


Figure A3. Relationships between the C/N ratio and organic C fractions of cattle manure compost (CaMC) and rice straw compost (RSC) samples. The functions for labile residue and total residues were fitted to these data.

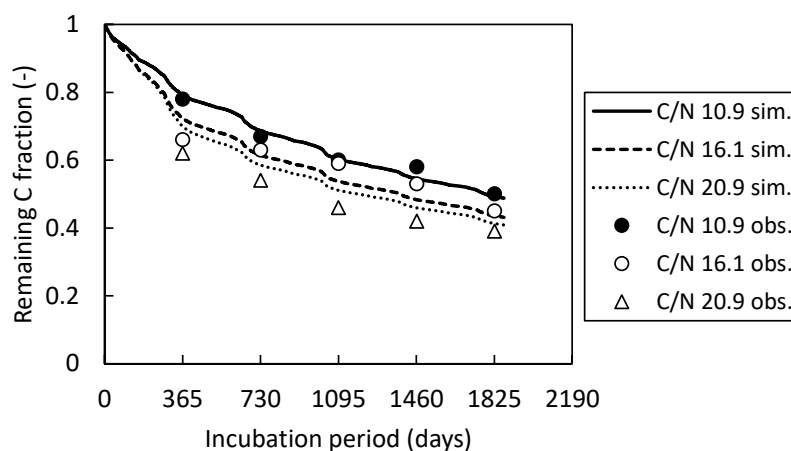


Figure A4. Decomposition of rice straw composts with different C/N ratios observed at a paddy site in Japan and simulated by the DNDC-Rice model.

Appendix A.3. Modeling of Biological N_2 Fixation

To simulate BNF in paddy soils, this study hypothetically assumed photosynthetic autotrophs as the N fixers that grow only under flooded conditions fixing N_2 and CO_2 at a constant ratio depending on light energy. Table A4 shows parameters, variables and equations of the BNF model developed in this study, where N_2 fixation rate by unit mass of N_2 fixers is determined by light energy available for unit mass of N_2 fixers. Irradiance at paddy surface, which is required to calculate N_2 fixation, is not usually measured or provided in meteorological databases open to the public. To run the BNF model, therefore, irradiance at paddy surface was calculated from the solar radiation and the leaf area index (LAI) according to Hasegawa[36].

Two parameters of the BNF model (V_{Nmax} and K_N) were optimized to reproduce net N_2 fixation observed by Hayashi et al. [15] on a paddy soil in Tsukubamirai, Japan. They conducted field incubation of submerged soil columns (62 mm in inner diameter) at the plot without N fertilizer application (0N plot) of the study site equipped with a free-air CO_2 enrichment (FACE) facility. The change in total N of the uppermost 1 cm soil layer was measured for 82 days in rice growing season. Although they measured N_2 fixation under both ambient and elevated CO_2 concentration (913 ppm

in average), this study employed only the data (average of 4 replicates) under ambient CO₂ for the model calibration, thus presently not accounting for the effect of elevated CO₂ on BNF.

At the same site, solar radiation was measured for the entire rice growing season of 2012[37]. After the transplanting in 2012, LAI of the rice cultivar Koshihikari was measured 6 times in the plot with fertilization of 8 g N m⁻² (8N plot), but only 3 times in the 0N plot where N fixation was measured. To get LAI data as precise as possible, therefore, we interpolated LAI in the 0N plot by scaling that in the 8N plot by a factor of 0.6 (Fig. A5A). Based on the solar radiation and LAI in the 0N plot, irradiance at paddy surface was calculated for the rice growing season. For the 82 days of soil column incubation (May 28 to August 18), irradiance at paddy surface summed up to 794 MJ m⁻² (Fig. A5B), whereas sum of solar radiation was 1513 MJ m⁻². With these inputs of irradiance at paddy surface, V_{Nmax} and K_N were optimized by the Downhill Simplex method [38] to minimize the root mean squared error (RMSE) of calculated N₂ fixation, resulting in R² of 0.991 (Fig. A5B).

The calibrated BNF model was built into DNDC-Rice model to calculate BNF during flooded period, based on input of solar radiation and simulated LAI. The C/N ratio of N₂ fixers biomass was assumed to be 6.0 g g⁻¹, referring to the ratio of total C increase to total N increase observed in the field soil incubation[15].

Table A4. Functions to simulate biological N₂ fixation in paddy soil.

Function/variable/parameter	Unit	Equation	Reference
Maximum specific N fixation rate	g N g ⁻¹ N day ⁻¹	$V_{Nmax}=3.346$ (optimized)	
Specific irradiance saturation constant	10MJ g ⁻¹ N day ⁻¹	$K_N=48.09$ (optimized)	
Initial N of living N fixers	kg N ha ⁻¹	$N_{F0}=0.0001$ (fixed)	
Decay rate constant of living N fixers	day ⁻¹	$\beta=0.01$ (fixed)	
Solar radiation	MJ m ⁻² day ⁻¹	I_0 (Variable)	
Leaf area index	m ² m ⁻²	F (Variable)	
Irradiance at paddy surface	MJ m ⁻² day ⁻¹	$I=I_0e^{-kF}$ $k=0.55$ (fixed)	[36]
N of living N fixers	kg N ha ⁻¹	N_F (variable)	
Specific irradiance per N of living N fixers	10MJ g ⁻¹ N day ⁻¹	$I_N=I/N_F$	
N fixation rate	kg N ha ⁻¹ day ⁻¹	$G_N=N_FV_{Nmax}\frac{I_N}{K_N+I_N}$	
Daily change in N of living N fixers	kg N ha ⁻¹ day ⁻¹	$\Delta N_F=G_N-\beta N_F$	

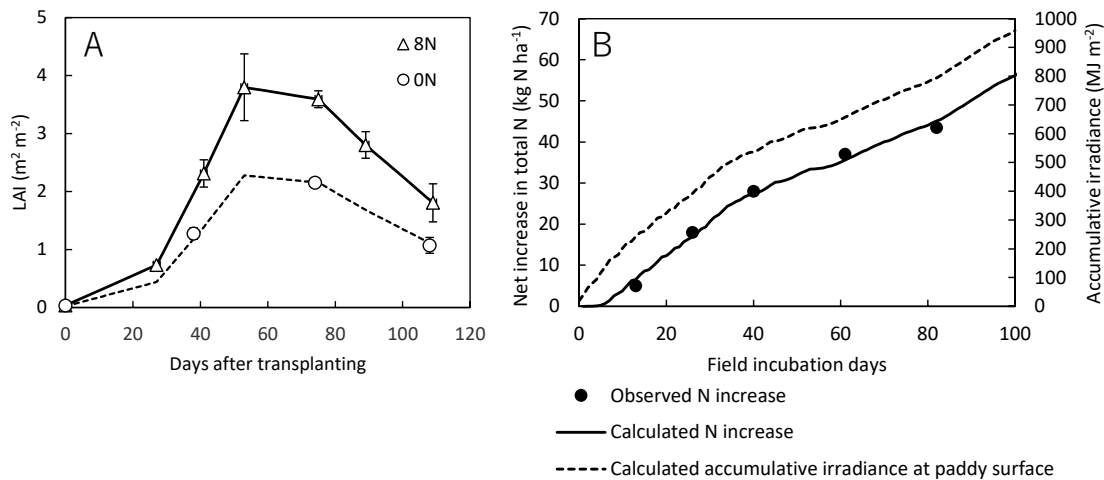


Figure A5. LAI of the rice cultivar Koshihikari in the 8N and 0N plots at the Tsukubamirai site in 2012. LAI in the 0N plot was fitted by scaling the LAI measured in the 8N plot (A). Observed and calculated total N increase (N₂ fixation) during the soil incubation experiment in the 0N plot at the Tsukubamirai site in 2012. Calculated accumulative irradiance at the paddy surface is shown as well (B).

Appendix A.4. Modeling of Nitrification and Denitrification

To simulate nitrification in paddy soils, DNDC-Rice assumes autotrophic aerobic nitrification of dissolved NH₃ [39,40]. Table A5 summarizes the functions to simulate nitrification. Hourly nitrification rate depends on temperature, pH, NH₄⁺ concentration, soil O₂, and nitrifiers biomass. In the nitrification process, fixed portion of nitrified N is assumed to be lost as NO and N₂O.

When NH₄⁺ has been oxidized to NO₃⁻, it can be denitrified under anaerobic conditions. The denitrification model in DNDC-Rice (Table A6) follows that in DNDC [12,14] except that DNDC-Rice quantitatively tracks electron transfer in each reduction and oxidation process as described by Fumoto et al.[4]. The hourly specific growth rate of NO_x denitrifiers is determined depending on NO_x concentration, DOC concentration, temperature, and pH. Then, the model calculates the hourly rates of NO_x reduction and DOC consumption required for growth and maintenance of denitrifiers biomass. To simulate emissions of NO, N₂O and N₂ from soil to atmosphere, hourly emission coefficient is calculated based on the clay content and air-filled porosity in the soil (Table A7).

Table A5. Functions to simulate nitrification in soils.

Function/variable/parameter	Unit	Equation	Reference
Temperature factor	-[12,14	$f_T = \left(\frac{60 - T}{25.78}\right)^{3.503} e^{\frac{3.503(T-34.22)}{25.78}}$	[14]
Affinity constant for dissolved NH ₃	mol L ⁻¹	$K_{NH3} = 26.0 \times 10^{-6}$	[39]
Dissolved concentration	NH ₃ mol L ⁻¹	$[NH_3] = \frac{[NH_4^+][OH^-]}{1.8 \times 10^{-5}}$	
Maximum nitrification rate specific to nitrifiers biomass	g N g ⁻¹ C h ⁻¹	$\mu_{NIT} = 1.68$	[41]

Nitrifiers biomass in soil	kg C kg ⁻¹	B_{NIT} (variable)	
Nitrification rate in soil	kg N kg ⁻¹ h ⁻¹	$= f_T \mu_{NIT} \frac{[NH_3]}{K_{NH_3} + [NH_3]} B_{NIT} \frac{[O_2]}{[O_2]_{max}}$ [O₂] = O₂ concentration in soil water (mol L⁻¹) [O₂]_{max} = O₂ concentration in equilibrium with atmosphere (mol L⁻¹)	
Growth yield of nitrifiers	g C g ⁻¹ N	$Y_{NIT} = 0.095$	[40]
Nitrifiers death rate	kg C kg ⁻¹ h ⁻¹	$0.0003 f_T B_{NIT}$	[14]
Net growth rate of nitrifiers biomass	kg C kg ⁻¹ h ⁻¹	$G_{NIT} = Y_{NIT} R_{NIT} - 0.0003 f_T B_{NIT}$	
N/C ratio in nitrifiers	g N g ⁻¹ C	0.29	[12]
NH ₄ uptake for nitrifiers growth	kg N kg ⁻¹ h ⁻¹	$0.29 G_{NIT}$	
Nitrification-induced NO production	kg N kg ⁻¹ h ⁻¹	$0.0025 f_T R_{NIT}$	[14]
Nitrification-induced N ₂ O production	kg N kg ⁻¹ h ⁻¹	$0.0024 R_{NIT}$	[14]

Table A6. Functions to simulate denitrification in soils.

Function/variable/parameter	Unit	Equation
Effect of temperature	-	$f_T = 2^{\frac{(T-22.5)}{10.0}}$ $0 \leq f_T \leq 1$
Effect of pH on NO _x reduction	-	$f_{PH} = 2 \left(1 - \frac{1}{1 + \exp\left(\frac{pH - 4.25}{0.5}\right)} \right) \text{ for NO}_3^-$ $f_{PH} = 2 \left(1 - \frac{1}{1 + \exp(pH - 5.25)} \right) \text{ for NO}_2^- \text{ and NO}$ $f_{PH} = 2 \left(1 - \frac{1}{1 + \exp\left(\frac{pH - 6.25}{1.5}\right)} \right) \text{ for N}_2\text{O}$ $0 \leq f_{PH}$
Effect of NO _x concentration (kg N m ⁻³)	-	$f_{NO_x} = \frac{[NO_x]}{0.083 + [NO_x]}$
Effect of DOC concentration (kg C m ⁻³)	-	$f_{DOC} = \frac{[DOC]}{0.017 + [DOC]}$

Maximum specific growth rate of denitrifiers on NO _x	h ⁻¹	$m_{NO3} = 0.67, m_{NO2} = 0.67,$ $m_{NO} = 0.34, m_{N2O} = 0.34$
Specific growth rate of denitrifiers on NO _x	h ⁻¹	$\mu_{NOx} = m_{NOx}f_{NOx}f_{DOC}f_Tf_{PH}$
Growth yield of denitrifiers on NO _x	g C g ⁻¹ N	$Y_{NO3} = 0.401, Y_{NO2} = 0.428, Y_{NO} = 0.151, Y_{N2O} = 0.151$
Growth yield of denitrifiers on DOC	g C g ⁻¹ C	$Y_{DOC} = 0.503$
Maintenance coefficient of denitrifiers on NO _x	g N g ⁻¹ C h ⁻¹	$M_{NO3} = 0.09, M_{NO2} = 0.0349, M_{NO} = 0.0792, M_{N2O} = 0.0792$
Maintenance coefficient of denitrifiers on DOC	g C g ⁻¹ C h ⁻¹	$M_{DOC} = 0.0076$
Denitrifiers biomass in soil	kg C kg ⁻¹	B_{DN} (variable)
NO _x reduction rate for growth and maintenance of denitrifiers	kg N kg ⁻¹ h ⁻¹	$R_{NOx} = \left(\frac{\mu_{NOx}}{Y_{NOx}} + M_{NOx} \frac{[NO_x]}{[N]} f_T f_{PH} \right) B_{DN}$ $[N] = \text{sum of NO}_x \text{ concentrations (kg N m}^{-3}\text{)}$
Denitrifiers growth rate	kg C kg ⁻¹ h ⁻¹	$G_{DN} = (\mu_{NO3} + \mu_{NO2} + \mu_{NO2} + \mu_{N2O}) B_{DN}$
Denitrifiers death rate	kg C kg ⁻¹ h ⁻¹	$f_T M_{DOC} Y_{DOC} B_{DN}$
N/C ratio in denitrifiers	g N g ⁻¹ C	0.29

Table A7. Functions to simulate NO_x gas emissions.

Function/variable/parameter	Unit	Equation	Reference
Gas emission coefficient of NO _x	h ⁻¹	$P_{NO} = P_{N2O} = 0.0006 + 0.0013AD$ $+ PA(0.013 - 0.005AD)$ $P_{N2} = 0.0017 + PA(0.025 - 0.0013AD)$ $0 \leq P_{NOx} \leq 1$ $AD = \frac{Clay}{0.315}$ $Clay = \text{clay content in soil (g g}^{-1}\text{)}$ $PA = \text{air-filled porosity in soil (m}^3 \text{ m}^{-3}\text{)}$	[12,14]

Appendix A.5. Modeling of Growth of Main Rice and Ratoon Rice

DNDC-Rice, like other versions of the DNDC model, includes a relatively simple crop growth model that represents crop phenological stage with the Plant Growth Index (PGI), based on the effective temperature sum with the base temperature at 10 °C (TDD₁₀). Another important crop specific parameter is the maximum N uptake that refers to how much N the crop absorbs under the optimal condition with ample N supply without any stress.

To calibrate parameters controlling biomass partitioning and leaf area development, we employed a 10-year monitoring dataset on the cultivar Koshihikari grown at a rice paddy site called “Mase” in central Japan (36° 03’ N, 140° 01’ E). The Mase site was established on a farmer’s rice field in 1999 for continuous measurements of energy and CO₂ exchange fluxes by the eddy covariance methodology[42]. In each year, the cultivar Koshihikari was grown with locally typical managements. Along with the meteorology and flux measurements, rice plant was periodically (mostly every other week) sampled and analyzed for dry weight of each organ (root, stem, green leaf, dead leaf and panicle) and the leaf area index (LAI). For the period of 2001-2010, the average TDD₁₀ from transplanting to harvest was 1644.0 (S.D. 94.2) °C days. Based on the dataset, we approximated following 3 parameters as functions of PGI (defined as $PGI = TDD_{10}/1644.0$): biomass fraction of panicle (FPA), leaf/straw ratio (LSR), and specific leaf weight (SLW) (Fig. A6, Table A8).

Based on the findings on ratoon rice growth mentioned in the main text, we built a sub model in DNDC-Rice that simply relates N demand of ratoon rice to the effective temperature sum from the day of main rice harvest (Table A9). Similarly to the main rice, ratoon rice was assumed to terminate its growth at TDD₁₀ of 1200 or 1600 °C days. In addition to N uptake from soil, this model calculates translocation of N and C from stubbles to ratoon. As only limited information was available to quantify what portion of stubble N and C can be translocated to ratoon, we provisionally assumed that 25% of stubble N at harvest can be translocated, referring to the literature data of nonstructural carbohydrate concentration (27-30%) in Koshihikari stubble[21]. Ten % of N and C in stems and leaves at harvest were assumed to be left as stubble N and C.

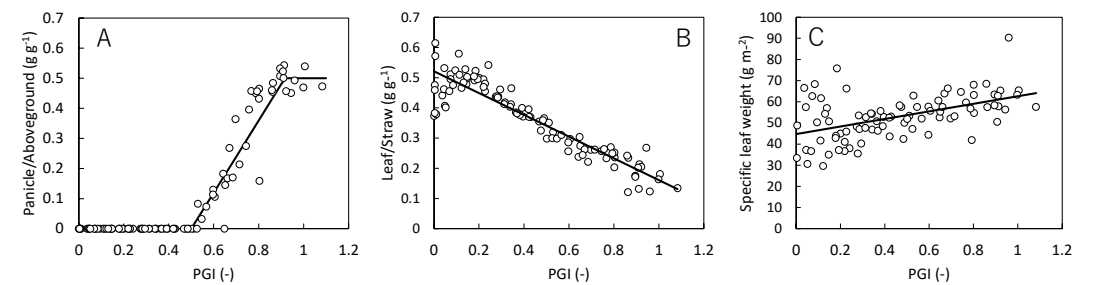


Figure A6. Observed data and fitted functions of the panicle/aboveground ratio (A), the leaf/straw ratio (B) and the specific leaf weight (C) at the Mase rice paddy site for 2001-2010.

Table A8. Functions to simulate main rice growth.

Parameter/variable	Unit	Equation
Daily mean air temperature	°C	T_a (Variable)
Effective temperature sum from transplanting	°C days	$TDD_{10} = \sum \max(T_a - 10, 0)$
Effective temperature sum for maturity	°C days	TDD_{10max} (Calibrated)
Plant growth index	-	$PGI = \frac{TDD_{10}}{TDD_{10max}}$
Maximum N uptake	kg N ha ⁻¹	N_{max} (Calibrated)
Daily demand for inorganic N	kg N ha ⁻¹ day ⁻¹	$ND = N_{max} \frac{\max(T_a - 10, 0)}{TDD_{10max}},$ $TDD_{10} \leq TDD_{10max}$

C/N ratio of main rice	g g^{-1}	50
Biomass fraction of root	g g^{-1}	$FRT = 0.09 \quad (PGI < 0.5)$ $FRT = 0.05 + 0.08(1 - PGI) \quad (PGI \geq 0.5)$
Biomass fraction of panicle		$FPA = 0 \quad (PGI < 0.5)$ $FPA = \min(1, 1.2(PGI - 0.5))(1 - FRT) \quad (PGI \geq 0.5)$
Biomass fraction of straw		$FST = 1 - FRT - FPA$
Leaf/straw ratio	g g^{-1}	$LSR = -0.3598PGI + 0.5208$
Specific leaf weight	g m^{-2}	$SLW = 17.951PGI + 44.749$
Rooting depth	m	$ZRT = \min(0.3, 0.05 + 0.01DAT)$ $DAT = \text{days after transplanting}$

Table A9. Functions to simulate ratoon rice growth.

Parameter/variable	Unit	Equation
Daily mean air temperature	$^{\circ}\text{C}$	T_a (Variable)
Effective temperature sum from harvest	$^{\circ}\text{C days}$	$TDD_{10} = \sum \max(T_a - 10, 0)$
Effective temperature sum for maturity	$^{\circ}\text{C days}$	$TDD_{10max} = 1600$
Maximum N uptake by ratoon rice	kg N ha^{-1}	RN_{max} (Calibrated site specifically)
Daily demand for inorganic N	$\text{kg N ha}^{-1} \text{ day}^{-1}$	$RND = RN_{max} \frac{\max(T_a - 10, 0)}{TDD_{10max}},$ $TDD_{10} \leq TDD_{10max}$
C/N ratio of ratoon rice	g g^{-1}	30
Portion of stubble translocated to ratoon	kg N ha^{-1}	$N_{reserve} = 0.25N_{stubble}$ $N_{stubble} = \text{main rice stubble N at harvest (kg N ha}^{-1}\text{)}$
Daily translocation of stubble N to ratoon	$\text{kg N ha}^{-1} \text{ day}^{-1}$	$N_{trans} = 0.2N_{reserve}$
Daily translocation of stubble C to ratoon	$\text{kg C ha}^{-1} \text{ day}^{-1}$	$C_{trans} = 30N_{trans}$
Active root depth of ratoon rice	m	0.2

Appendix A.6. Calibration of the Growth Model for Main Rice and Ratoon Rice

The parameters TDD_{10max} and N_{max} for main rice can be cultivar specific. Referring to the TDD_{10} from transplanting to harvest at each site, TDD_{10max} was set at 1200 °C days for Nanatsuboshi (the HO1 and HO2 sites), and 1600 °C days for the other cultivars (at the AO3, NI4, SH5 and SH6 sites). N_{max} was set at 150 kg N ha⁻¹ for all cultivars, based on the highest value of measured N uptake.

To reproduce observed ratoon rice growth, the maximum N uptake by ratoon rice, RN_{max} , was calibrated site specifically so that the RMSE of estimated ratoon N was under 3.0 kg N ha⁻¹ at each site (Table A10, Fig. A7). Consequently, RN_{max} at the AO3 site was adjusted at a high value (150 kg N ha⁻¹) to reproduce the rapid growth observed for just 13 days in 2023.

Table A10. Growth parameters to simulate main rice and ratoon rice growth at the monitoring sites.

Site	Main rice	Ratoon rice					
	Cultivar	TDD ₁₀ for maturity	Maximum N uptake	C/N	TDD ₁₀ for maturity	Maximum N uptake	C/N
		(°C days)	(kg N ha ⁻¹)	(g g ⁻¹)	(°C days)	(kg N ha ⁻¹)	(g g ⁻¹)
HO1, HO2	Nanatshuboshi	1200	150	50	1200	20	30
AO3	Masshigura	1600	150	50	1600	150	30
	Harewatari	1600	150	50	1600	150	30
NI4	Koshihikari	1600	150	50	1600	40	30
SH5	Mizukagami	1600	150	50	1600	60	30
SH6	Koshihikari	1600	150	50	1600	28	30

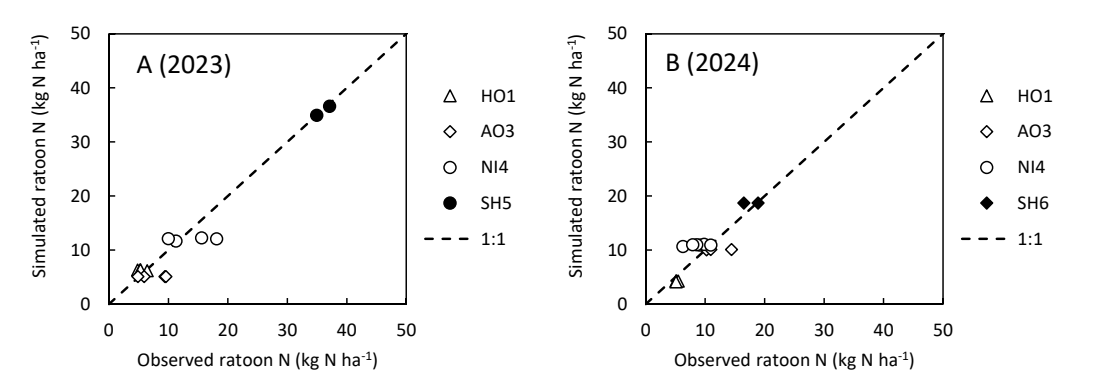


Figure A7. Ratoon rice N observed and simulated at the monitoring sites in 2023 (A) and 2024 (B). Observed data are the average for each treatment.

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