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Changes in the Nitrogen Budget and Soil Nitrogen in a Field with Paddy–Upland Rotation with Different Histories of Manure Application

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Academic Editor: Ryusuke Hatano
Received: 27 February 2017; Accepted: 25 April 2017; Published: 28 April 2017

Abstract: In northern Japan, declines in soil nitrogen fertility have occurred in paddy–upland rotation systems with soybean cultivation. A six-year lysimeter experiment was conducted to evaluate the nitrogen budget in paddy–upland rotation (three-year for upland soybean, then three-year for flooded paddy rice) and to clarify the effect of preceding compost application (immature or mature compost over four consecutive years of forage rice cultivation) on the nitrogen budget and soil nitrogen fertility. Available soil nitrogen throughout the experimental period and soybean and rice yields in both compost application plots tended to be higher than those in the control plot. The nitrogen budgets during both soybean and rice cultivation were negative, and the amount of nitrogen loss in both compost application plots tended to be higher than that in the control plot. The nitrogen loss during rice cultivation (-2.3 to -4.3 g N m^{-2} year^{-1}) was less than that during soybean cultivation (-9.6 to -14.6 g N m^{-2} year^{-1}). Nitrogen loss estimated based on the nitrogen budget agreed well with that estimated based on changes in soil nitrogen storage during soybean cultivation but not during rice cultivation, suggesting underestimation of nitrogen loss from the rice paddy.

Keywords: nitrogen budget; paddy–upland rotation; preceding compost application; flooded paddy rice; upland soybean

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1. Introduction

In Japan, rice production has been adjusted over 40 years due to decrease in domestic consumption. The area of paddy fields in Japan has continued to decline at a rate of about 20,000 ha^{-1} year^{-1}, and the total area was 2,355,000 ha in 2010. Around 70% of the paddy area was cultivated with paddy rice in summer, and the rest was cultivated with upland crops after rice paddy fields were converted to upland fields or abandoned [1]. Recently, the paddy–upland rotation system, which alternates every few years between paddy rice cultivation and upland crop cultivation in drained paddy fields, has become a popular practice. Soybean is the main crop cultivated in the converted upland fields in Japan.

Recently, depletion of available soil nitrogen and a subsequent decline in soybean yield in upland fields in the repeated paddy–upland rotation system have been reported in northern Japan [2]. In addition, a decrease in available soil nitrogen (including soil nitrogen mineralization) with an increase in upland frequency (i.e., the number of years in soybean cultivation per total cultivation years) has been reported in fields with paddy–upland rotation [3–5]. Although soybean plants obtain nitrogen via symbiotic N_2 fixation in root nodules, they also take up significant soil nitrogen to meet their high nitrogen requirement [6,7]. Therefore, the nitrogen budget of soybean cultivation in a rotated paddy field could be negative, indicating nitrogen loss from the field. In fact, negative nitrogen budgets were reported recently in Japanese soybean-cultivated upland fields during the first year after conversion from paddy fields (-10.0 g N m^{-2} year^{-1} in Akita [8], -4.0 g N m^{-2} growing period^{-1} in Shiga [9]).

In general, the nitrogen budget in a rice paddy field is considered to be neutral [10] or slightly positive (accumulation) [11], suggesting that the marked field nitrogen loss from a rotated paddy field occurs during soybean cultivation. However, the nitrogen budget in rice paddy fields was reported to vary widely depending on field management practices [12]. To our knowledge, there have been no detailed reports on the nitrogen budget in a rice-cultivated rotated paddy field. Therefore, to develop an efficient system for maintaining soil nitrogen fertility in rotated paddy fields, it is essential to evaluate the nitrogen budget in rotated paddy fields during both the soybean and rice cultivation periods.

Thus, the objective of this study was to investigate the details of the nitrogen budget in rotated paddy fields based on our previous study in the first year of soybean cultivation [8]. The validity of the estimated nitrogen budget was evaluated by comparison with the change in soil nitrogen storage. In addition, to clarify the effects of soil nutrient availability on crop yield and the field nitrogen budget, measurements were made in fields with different histories of compost application.

2. Materials and Methods

2.1. Experimental Field

The experiment was conducted in the lysimeter plots of the Akita Prefectural Agricultural Experiment Station (39°35′ N, 140°12′ E) for six years (June 2008 to May 2014). Three lysimeter plots (each 15 m^2 by 2 m deep) were filled with gray lowland soil (Eutric Fluvisols; Food and Agriculture Organization/UNESCO) in 2000 and with drainage pipes buried at a depth of 60 cm from the soil surface in 2002. Detailed information about the studied lysimeter fields including soil properties was given in our previous paper [8]. The mean annual temperature and precipitation recorded by the automated meteorological data acquisition system (AMeDAS), 5 km from the experimental field (Yuwa, 39°37′ N, 140°13′ E) were 10.9 °C and 1775 mm, respectively (Table 1). In this region, snowfall generally occurs from December to March. In the study period, air temperature during the cultivation period (May–September) in 2010 and 2012 was higher than the average, whereas monthly precipitation in 2008 and 2012 was lower than the average.

2.2. History of Compost Application

Prior to this study, forage rice (*Oryza sativa* L. cv. Bekoaoba) was cultivated under paddy field conditions for four consecutive years (2004–2007). During the cultivation, 3.0 kg m^{-2} (as fresh matter) of immature or mature compost made of livestock manure (C/N ratios: 18.2–24.3 and 10.0–16.9, respectively) was applied to the plots (i.e., immature compost or mature compost plots, respectively) each year. In the control plot, forage rice was cultivated without compost application. The application rates of nitrogen, phosphorus, and potassium by compost and chemical fertilizer are listed in Table 2. At the yellow ripening stage (mid-September) of each year, all aboveground biomass except for stubble (approximately 5 cm from the soil surface) was harvested and removed from the plots.

Table 1. Monthly air temperature and precipitation.

Month	Air Temperature (°C)								Precipitation (mm)							
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Average** [†]	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Average** [†]
Jan.	−1.7	0.0	−0.3	−2.5	−2.4	−2.3	−1.4	−0.7	93	199	165	93	54	71	129	135
Feb.	−1.3	0.0	−0.9	−0.1	−2.5	−1.8	−1.4	−0.4	67	140	73	67	64	51	67	90
Mar.	4.2	2.8	1.8	0.9	2.0	2.1	2.0	2.4	66	124	119	82	180	80	126	101
Apr.	10.4	8.8	6.8	7.4	8.4	7.0	8.4	8.3	39	121	142	155	103	141	26	119
May	14.2	15.0	13.4	13.5	14.2	13.5	14.5	14.2	90	82	154	181	85	64	127	114
Jun.	18.3	18.8	19.6	18.3	18.3	20.2	20.1	19.0	53	110	174	294	69	22	174	118
Jul.	23.0	21.7	23.8	24.0	22.4	22.3	23.0	21.9	155	342	246	81	176	544	152	197
Aug.	22.6	22.6	25.8	24.2	25.3	24.0	23.3	23.7	246	169	234	243	64	250	277	211
Sep.	19.6	18.1	20.1	20.3	22.6	19.7	18.2	19.6	62	80	207	386	76	184	155	150
Oct.	13.8	12.9	13.6	13.2	13.8	14.3	12.1	13.1	165	167	143	147	190	241	213	169
Nov.	6.6	7.5	7.1	8.1	6.5	5.6	7.5	7.3	240	232	243	139	388	358	130	209
Dec.	2.6	1.7	2.5	0.4	−0.1	1.5	0.0	1.9	153	138	161	124	158	174	221	162
Annual [‡]	11.0	10.8	11.1	10.6	10.7	10.5	10.5	10.9	1426	1903	2059	1989	1605	2178	1794	1775
May-Sep. [‡]	19.5	19.2	20.5	20.0	20.6	20.0	19.8	19.7	605	782	1015	1184	470	1063	884	791

[†] 2003–2010 (8 years). [‡] Average for air temperature and total for precipitation.

Table 2. Cultivation history and amount of applied nutrient before (2000–2007) and during the study period (2008–2013).

Year	Crop	Cultivar	T-N (g m^{-2})					T-P (g m^{-2})					T-K (g m^{-2})					Remarks
			C	**I**		**M**		**C**	**I**		**M**		**C**	**I**		**M**		
			CF	**CF**	**LMC**	**CF**	**LMC**	**CF**	**CF**	**LMC**	**CF**	**LMC**	**CF**	**CF**	**LMC**	**CF**	**LMC**	
2000	Soybean	NO	NO	NO	-	NO	-	NO	NO	-	NO	-	NO	NO	-	NO	-	Newly established
2001	Bare soil (upland)	NO	NO	NO	-	NO	-	NO	NO	-	NO	-	NO	NO	-	NO	-	
2002	Young soybean	NO	NO	NO	-	NO	-	NO	NO	-	NO	-	NO	NO	-	NO	-	
2003	Soybean	NO	NO	NO	-	NO	-	NO	NO	-	NO	-	NO	NO	-	NO	-	
2004	Forage rice	Bekoaoba	10.0	10.0	18.6	10.0	15.6	2.6	2.6	17.9	2.6	24.5	5.0	5.0	NO	5.0	NO	3 kg FM m^{-2} of immature and mature compost for I and M, respectively every year
2005			12.0	12.0	11.4	12.0	23.1	2.6	2.6	6.7	2.6	15.5	5.0	5.0	17.9	5.0	31.3	
2006			8.5	8.5	12.1	8.5	22.9	2.6	2.6	5.9	2.6	15.9	5.0	5.0	13.9	5.0	30.5	
2007			10.0	10.0	16.5	10.0	22.7	2.6	2.6	9.6	2.6	16.8	5.0	5.0	15.7	5.0	28.6	
2008	Soybean	Ryuho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	This study
2009			2.0	2.0	-	2.0	-	2.6	2.6	-	2.6	-	5.0	5.0	-	5.0	-	
2010			2.0	2.0	-	2.0	-	2.6	2.6	-	2.6	-	5.0	5.0	-	5.0	-	
2011	Staple rice	Yumeobako	3.0	3.0	-	3.0	-	0.9	0.9	-	0.9	-	1.7	1.7	-	1.7	-	This study
2012		Akitakomachi	8.0	8.0	-	8.0	-	3.5	3.5	-	3.5	-	6.6	6.6	-	6.6	-	
2013			10.0	10.0	-	10.0	-	4.4	4.4	-	4.4	-	8.3	8.3	-	8.3	-	

C, control; CF, chemical fertilizer; I, immature compost; LMC, livestock manure compost; M, mature compost; NO, not obtained; T-K, total potassium; T-N, total nitrogen; T-P, total phosphorus.

2.3. Plant Cultivation

Based on a report that a rotation of 2–3 years for upland and paddy fields was effective for maintaining stable productivity in well-drained gray lowland soil [13], soybean under upland conditions and staple rice under flooded paddy conditions were each cultivated for three consecutive years. To clarify the effects of different histories of compost application on the soil nutrient availability, plant growth and nitrogen budget, same cultivation management were applied to all plots during the study period. No organic materials were applied to any plots throughout the six-year study period.

In 2008, the lysimeter plots were converted from paddy to upland conditions, and soybean (*Glycine max* (L.) Merr. cv. Ryuho) was cultivated for three years (2008–2010). Based on the cultivation guidelines for this region [14], chemical fertilizer was applied at a rate of 0 or 2 g N m^{-2} to all plots as a basal fertilizer in 2008 or 2009 and 2010, respectively (Table 2). In all plots and years, no top-dressing of fertilizer was applied. Soybean seeds were stripe-sown at a density of 10.9 plants m^{-2} in early June. Ridging was conducted once at the end of June or July. Chemical treatments to control diseases and pests were conducted according to local conventions. Harvest was carried out in early October. Plant residue after harvesting from each plot was subsequently scattered across the same plot.

In 2011, the lysimeter plots were converted from upland to paddy conditions, and staple rice was cultivated for three years (*O. sativa* cv. Yumeobako in 2011 and cv. Akitakomachi in 2012 and 2013). In mid-May, plowing, basal fertilizer application, submerging, and puddling were conducted. Based on the culture guidelines for this region [15], chemical fertilizer was applied at a rate of 0 or 6 g N m^{-2} to all plots as a basal fertilizer in 2011 or 2012 and 2013, respectively (Table 2). In late May, 35-day-old rice seedlings were transplanted to the lysimeter plots with four seedlings per hill, at a density of 20.8 hills m^{-2}. In late July, 3 or 2 g N m^{-2} of chemical fertilizer (2011 or 2012 and 2013, respectively) was applied as top-dressing. During the flooding period before mid-season drainage, flooding water depth was kept around 3–5 cm by irrigating with water from a farm pond near the experiment station and draining surface water via a drain outlet near the soil surface (mainly at the time of high water level after a heavy rain, mid-season and final drainages). Mid-season drainage occurred from late June to mid-July, depending on plant growth and field moisture conditions. Intermittent drainage was subsequently performed after the mid-season drainage, and final drainage was carried out in late August. Plants were harvested in late September. After harvesting, rice straw from each plot was scattered on that plot.

2.4. Soil Sampling and Analysis

To determine the change in soil nitrogen storage during the soybean and rice cultivation periods, bulk soil samples were taken at 0–10, 10–20, and 20–30 cm depths by using an auger (with three replications in each plot) before starting this study (November 2007), after three years of soybean cultivation (May 2011), and after three years of rice cultivation (April 2014). After the soil samples were air-dried, sieved (2-mm mesh), and finely ground, total nitrogen content was measured using an N/C analyzer (NC-900 and NC-22F, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). To eliminate the effect of preceding manure application on and temporal variation in soil bulk density, soil nitrogen storage (0–30 cm) was calculated on a soil mass basis [16,17]. In May 2008 (before plowing), intact soil samples corresponding to the upper layer (0–10 cm) and lower layer (10–30 cm) were taken by using a 100-cm^3 core sampler for bulk density measurement. To make the dry soil mass of the immature and mature compost plots equal to that of the control plot, their soil depths were adjusted. The calculated soil mass in May 2008 was used to calculate soil nitrogen storage at all sampling times. The bulk densities of the upper or lower layers corresponded to the total nitrogen contents at depths of 0–10 or 10–20 and 20–30 cm, respectively. The rate of decline in soil nitrogen storage was calculated based on the difference between each sampling.

To determine the content of available nitrogen, soil samples were collected from the surface layer (0–10 cm) of each plot before starting this study (April 2008) and after harvesting each year.

The air-dried and sieved (2-mm mesh) samples were incubated under flooded conditions at 30 °C for four weeks, and mineralized NH_4-N was quantified as available nitrogen [8,18].

2.5. Plant Growth, Nitrogen Accumulation, and Yield

2.5.1. Soybean

The amounts of accumulated nitrogen in soybean plants derived from symbiotic N_2 fixation and uptake by roots were determined following Takakai et al. [8] based on the relative ureide method [19,20]. Briefly, xylem sap was collected from five consecutive soybean plants with moderate growth by cutting their stem at the basal part (approximately 1 cm below the cotyledon node) at the V3 (third unrolled trifoliolate leaf, early July), R1 (beginning bloom, early August), R4 (full pod, late August), and R6 (full seed, early September) stages [21], where V and R indicate the vegetative and reproductive stages, respectively. Soybean plant samples after xylem sap collection were separated into leaves, stems, petioles, pods, roots, and nodules and then dried at 80 °C; after measuring the dry-matter weight, the samples were ground for the measurement of nitrogen content using the N/C analyzer. The total amount of accumulated nitrogen in soybean plants was calculated by multiplying the dry-matter weight by the nitrogen content. The total amount of accumulated nitrogen in soybean plants at the R8 stage (full maturity: harvesting, early October) was also determined as the sum of nitrogen in grains (described below), harvesting reside (pods, stems and roots) and litter (mainly fallen leaves and petioles) [8]. By determining the concentrations of N compounds in the xylem sap of soybeans, the accumulated N in plant was divided into symbiotic N_2 fixation by nodules (ureide-N: allantoin and allantoic acid) and uptake by roots (NO_3-N and amino-N: asparagine and aspartic acid). The nitrogenous compounds in the xylem sap were analyzed using capillary electrophoresis (P/ACE MDQ, Beckman Coulter, Brea, CA, USA) following Sato et al. [22,23].

At the harvesting stage (R8, early October), yield (5.5-mm sieved) and yield components of soybean (number of pods, number of grains, and 100-grain weight) were determined (for further details, see [8]). The amount of nitrogen in harvested grain was also determined.

2.5.2. Rice

Yield and yield components of rice were determined as described by Takakai et al. [24]. At the harvesting stage (late September), 72 rice hills were harvested from each plot to measure the yield of brown rice (1.9-mm sieved) and 1000-kernel weight. At the same time, plants from seven rice hills showing moderate growth in each plot were also collected. Plants from four of these hills were used to measure number of spikelets per panicle and percentage of filled spikelets. Those from the other three hills were separated into panicles or leaves and stems. Their dry-matter weight and nitrogen content were determined as well as soybean plants described above.

2.6. Measurement of Other Nitrogen Flows and Calculation of Nitrogen Budget

Figure 1 illustrates the nitrogen flows in upland fields with soybean and in paddy fields with rice. The nitrogen budget was estimated as the difference between the total input nitrogen flow and the total output nitrogen flow. Positive and negative values indicate net nitrogen accumulation and loss in the field, respectively. Annual values (from the beginning of cultivation one year to the beginning of cultivation in the next) were used for bulk nitrogen deposition, nitrogen leaching, and N_2O emission. The measurement methods for each nitrogen flow component are described in the following sections.

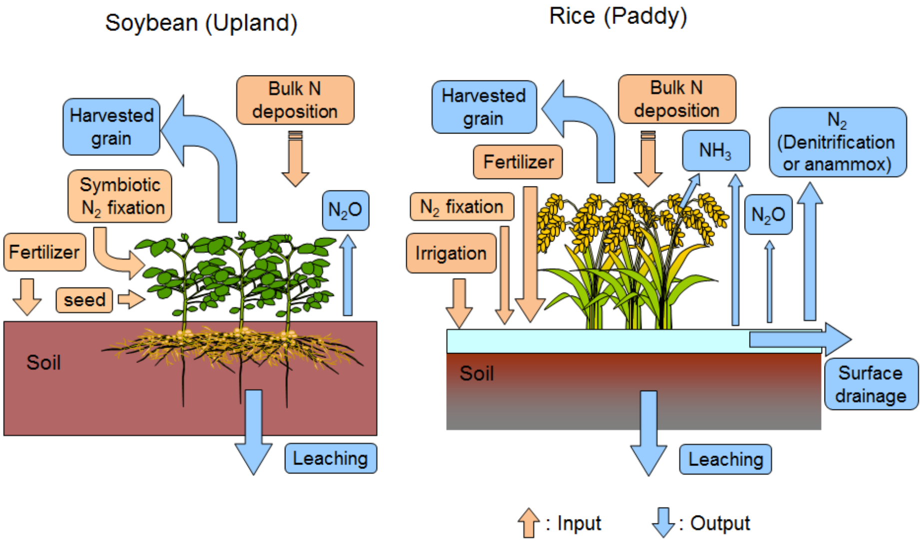

Figure 1. Outline of major nitrogen (N) flows in soybean upland field and rice paddy field. Anammox, anaerobic oxidation of ammonium; NH_3, ammonia volatilization; N_2, dinitrogen; N_2O, nitrous oxide. N_2 emission via denitrification in upland was not considered in this study.

2.6.1. Seeds and Seedlings

The nitrogen contents of soybean seeds and rice seedlings were determined in the same way as described previously for nitrogen contents in plants (Section 2.5). The nitrogen input in seeds and seedlings was calculated from these nitrogen contents, 100-grain weight (soybean only), and plant density.

2.6.2. Bulk Nitrogen Deposition

Samples of bulk rainfall (or snow) were collected using a 12.5-cm-diameter funnel (no snow period) or a 33-cm-diameter polyethylene bucket (snow period) installed in an open field adjacent to the lysimeter plots, with two replications. Total nitrogen concentration in a water sample was determined by the alkaline persulfate digestion method [8]. Briefly, a water sample was digested with alkaline persulfate solution (0.15 M $K_2S_2O_8$–0.1125 M NaOH) at 120 °C for 30 min. Thereafter, the sample was acidified with HCl (1 + 16) and its nitrate (NO_3-N) concentration was determined by ultra violet detection using a spectrophotometer (V-550; JASCO, Tokyo, Japan).

2.6.3. Irrigation

The amount of irrigation water was recorded and a water sample was collected at each irrigation event. Total nitrogen concentrations in the water samples were determined as well as bulk nitrogen deposition.

2.6.4. Nitrogen Leaching and Surface Drainage

During the soybean cultivation period and fallow period (i.e., upland condition), subsurface drainage was discharged freely through the drainage pipe buried at a 60-cm depth. The volume of drainage water was monitored using a tipping bucket with a pulse data logger (UIZ-TB200 and UIZ3639, respectively, UIZIN, Tokyo, Japan), except for 2008, when we directly measured the discharged volume before equipping the system [8]. Thereafter, a part of the discharged water was collected in a 240-L tank, from which water samples were taken as appropriate after a large rainfall event or during melting in the snow period.

During the rice cultivation period, subsurface drainage was discharged at a constant rate using a peristaltic pump (EW-07553-80, Cole-Parmer, Vernon Hills, IL, USA). The discharge rate was set as

1 mm day^{-1} by assuming downward leaching in a paddy field on gray lowland soil [25]. The sample of discharged water was collected weekly. At each surface drainage event, the amount of drained water was calculated based on the change in flooding water level and the water sample was collected. Total nitrogen concentrations of the water samples were determined as well as bulk nitrogen deposition.

2.6.5. N_2O Emission

A closed-chamber method was used to measure N_2O emissions [26]. Detailed information about the measurement was provided previously [8,27]. Briefly, cylindrical stainless-steel chambers (18.5–21.0 cm in diameter and 25 cm in height) were used for the measurements during the soybean cultivation and fallow periods without plants inside the chamber. Rectangular transparent acryl chambers (30 cm × 60 cm× 50 cm or 100 cm in length × width × height) were used for the measurements during the rice cultivation period including four hills of rice inside the chamber. The N_2O concentration of air sample which taken into a 10-mL vacuum vial was analyzed using a gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) equipped with an electron capture detector. The N_2O flux was calculated by a linear regression of increase in N_2O concentration with time. Measurements were made about once a week during the crop cultivation period and one to three times per month during the fallow period. The annual N_2O emission was calculated by integrating N_2O fluxes based on linear interpolation.

2.6.6. Other Flows

N flows that were not measured in this study were quoted from the literature. N input by N_2 fixation in rice paddy field was estimated to be 2.0 g N m^{-2} based on a measurement in a paddy field on gray lowland soils in Akita [28]. Nitrogen output by N_2 emission via denitrification and NH_3 volatilization in rice paddy field was calculated from the amount of nitrogen fertilizer application and emission factors by following the method of Katayanagi et al. [11]. Briefly, N_2 emission via denitrification was estimated as the sum of N_2 derived from fertilizer (25.8% of the fertilized N) and from the soil (0.38 g N m^{-2}) [29]. NH_3 volatilization was estimated as 1.4% of fertilized N [30]. Recently, some reports have pointed out the possibilities that anaerobic oxidation of ammonium (anammox) can be a significant process of nitrogen dynamics in a flooded paddy soil [31–33]. However, N_2 emission via anammox from rice paddy was not considered in this study due to their limitation in quantitative evaluation.

2.7. Statistical Analyses

All differences among the plots were compared by two-way analysis of variance (ANOVA, year × plot) followed by a Tukey test. In this study, differences with $p < 0.10$ were considered significant. For all statistical analyses, Excel Statistics 2012 for Windows (SSRI, Tokyo, Japan) was used.

3. Results

3.1. Changes in Soil Nitrogen

Assuming that soil nitrogen storage at a depth of 0–30 cm before compost application for forage rice cultivation (2004–2007) was similar among the plots, the application of immature and mature compost caused nitrogen storage to increase by 59 and 64 g N m^{-2}, respectively, compared to the control plot over the four years (Figure 2, Table S1). Especially, total nitrogen contents in surface soil (0–10 cm) in both compost application plots were higher than that in the control plot before the study. The decreases in the nitrogen storage during the three years of soybean cultivation in the immature and mature compost plots (46 and 32 g N m^{-2}, respectively) were higher than that in the control plot (27 g N m^{-2}). During the soybean cultivation, soil N storage in 0–10 cm decreased remarkably in both compost application plots, unlike the no change in the control plot. During the three years of rice cultivation, the decreases in the nitrogen storage in the immature and mature compost and control plots were 36, 31, and 27 g N m^{-2}, respectively. The decreases during rice cultivation were similar to, or slightly lower than, those during soybean cultivation. During the rice cultivation, soil N storage in 0–10 cm did not decrease in all plots, and tended to increase in the control and mature compost plots, while soil N storage in 10–30 cm decreased remarkably in all plots. The total decreases in the nitrogen storage during the six years of cultivation in the immature compost, mature compost and control plots were 82, 63, and 54 g N m^{-2}, respectively, meaning that 48% and 14% of the nitrogen increase from immature and mature compost application as compared to the control were lost during soybean and rice cultivation.

Before soybean and rice cultivation, available soil nitrogen in the immature and mature compost plots were almost 40 mg N kg^{-1} higher than that in the control plot (Figure 3). The available soil nitrogen in the control plot was almost steady around 90–100 mg N kg^{-1} during the soybean cultivation period. While, although the available soil nitrogen in both compost application plots decreased during the soybean cultivation period, the values were still 20 mg N kg^{-1} higher than in the control plot. The available soil nitrogen in all plots decreased after the first year of rice cultivation (2011) and tended to increase during the second and third years (2012 and 2013). After the six-year experiment, the available soil nitrogen contents in the immature and mature compost plots (131 and 149 mg N kg^{-1}) were still 22 and 40 mg N kg^{-1} higher than in the control plot (109 mg N kg^{-1}), respectively.

Figure 2. Decrease in soil nitrogen (N) storage (0–30 cm) under the soybean-rice cultivation.

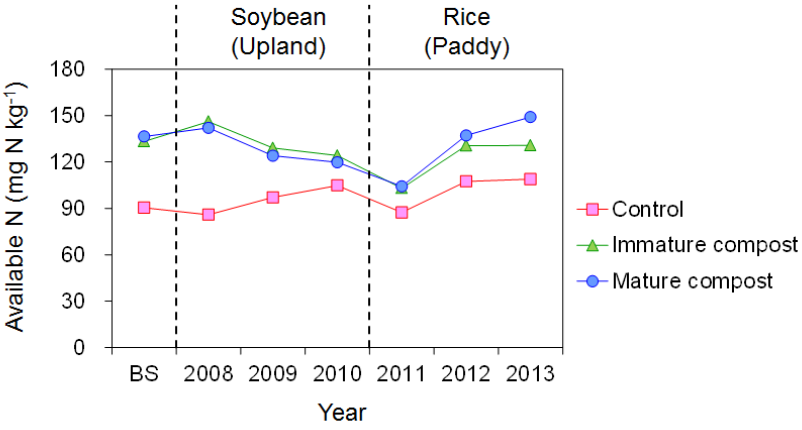

Figure 3. Inter-annual variation of available nitrogen (N) of the surface soil (0–10 cm) after cultivation of each year. BS, before the beginning of the study.

3.2. Plant Growth, Nitrogen Accumulation, and Yield

3.2.1. Soybean

Over the three years of soybean cultivation, the amount of nitrogen accumulation and nitrogen derived from root uptake and N_2 fixation ranged from 22.4 to 36.3, 7.6 to 18.5, and 13.4 to 27.4 g N m^{-2}, respectively (Figure 4). The amount of nitrogen accumulation in soybean in the mature compost plot was similar across the three years, whereas those values in the control and immature compost plots decreased between the first year (2008) and third year (2010). The decrease was mainly caused by the decrease in nitrogen accumulation during the late growth stage after R4 or R6 (Table S2). The amount of nitrogen accumulation in soybean increased in the following order: control plot < immature compost plot < mature compost plot, with a significant difference between the control and mature compost plots. The amount of nitrogen derived from N_2 fixation increased in the following order: immature compost plot < control plot < mature compost plot, and a significant difference was found between the immature and mature compost plots. The amount of nitrogen derived from root uptake did not differ significantly among the plots. Consequently, the final percentages of nitrogen accumulation derived from N_2 fixation at R8 were higher in the control and mature compost plots (65% and 64%, respectively) than that in the immature compost plot (55%) for three-year average. The percentages in the control plot remained relatively constant (60%–69%) across the three years, while the percentages in the mature compost plot in 2009 were lower than those in the other two years, due to decrease from R4 to R8 (Table S2).

Statistical analysis†

	C	I	M
N accumulation	a	ab	b
N uptake by root	a	a	a
N_2 fixation	ab	a	b

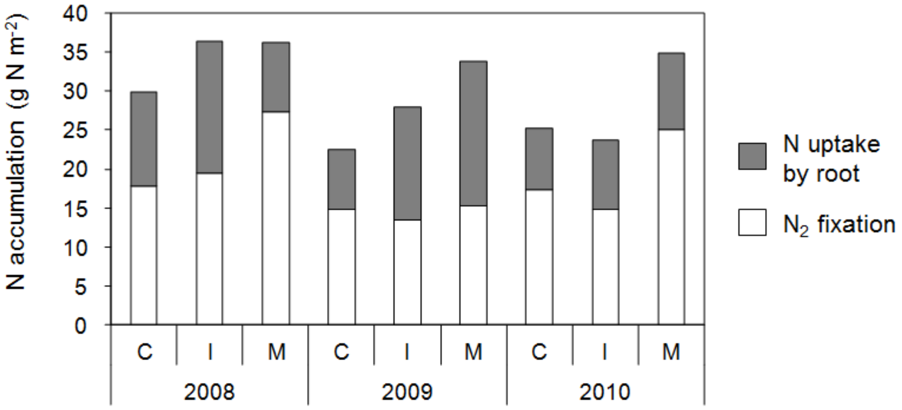

Figure 4. Amount of nitrogen (N) accumulation in soybean plant (cultivar: Ryuho) derived from dinitrogen (N_2) fixation and nitrogen uptake by root at harvesting stage for three years. † Different alphabets within a same line indicate significant difference among the plots (Two-way ANOVA (Year × Plot) followed by Tukey test, $p < 0.10$). C, control; I, immature compost; M, mature compost.

3.2.2. Rice

Over the three years of rice cultivation, the numbers of tillers in the immature and mature compost plots tended to be higher than that in the control plot (Table S3). In all three years, the amount of nitrogen accumulation of rice plants at the harvesting stage did not differ significantly among plots (Figure 5). The amount of nitrogen accumulation in the third year (2013: 7.7–10.3 g N m^{-2}) was lower than those in the other two years (12.4–14.2 g N m^{-2}) due to severe insect damage, especially to leaves.

Statistical analysis†	C	I	M
Total aboveground N accumulation	a	a	a

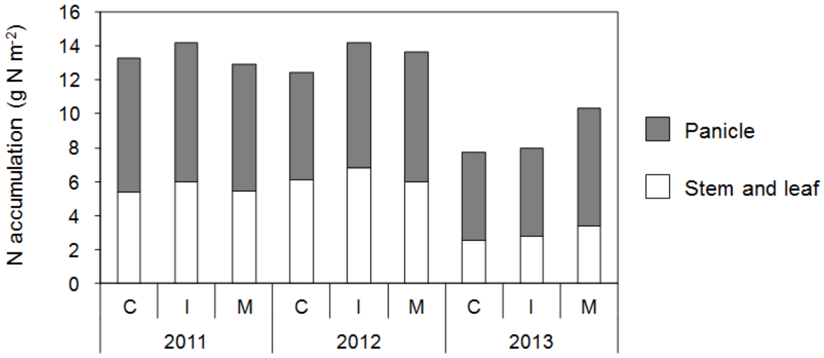

Figure 5. Amount of nitrogen (N) accumulation in rice plant (panicle or stem and leaf) at the harvesting stage for three years. Rice cultivar was Yumeobako (2011) and Akitakomachi (2012 and 2013). In 2013, rice plants were damaged severely by insects biting. † Different alphabets within a same line indicate significant difference among the plots (Two-way ANOVA (Year × Plot) followed by Tukey test, $p < 0.10$). C, control; I, immature compost; M, mature compost.

3.2.3. Yield

During the three years of soybean cultivation, the grain yields in the immature and mature compost plots (324–511 and 453–498 g m^{-2}, respectively) tended to be higher than that in the control plot (291–410 g m^{-2}), and the difference between the control and mature compost plots was significant (Figure 6, Table S4). The high yields in both compost application plots were mainly attributed to the high number of pods. The 100-grain weight in the third year (2010) was lower than those in the other two years and was higher in the mature compost plot than those in other two plots.

During the rice cultivation period, the grain yields increased in the following order: control plot < mature compost plot < immature compost plot, and a significant difference was found between the control and immature compost plots (Figure 6, Table S5). The yield increases were mainly attributed to the greater number of panicles. The rice grain yield in the third year (2013: 403–422 g m^{-2}) was lower than that in the second year (2012: 526–606 g N m^{-2}) when the same cultivar was planted, due to smaller numbers of panicles and filled spikelets and lower 1000-kernel weight.

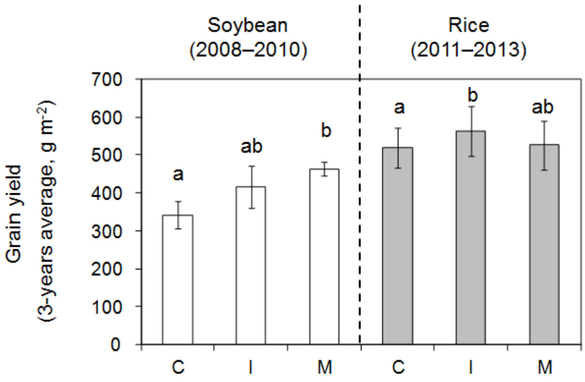

Figure 6. Effect of preceding compost application on the grain yield of soybean and rice (brown rice). Cultivar for soybean was Ryuho and for rice was Yumeobako (2011) and Akitakomachi (2012 and 2013). All yields were expressed on a 15% moisture content basis. Different alphabets within a same crop indicate significant difference in each nitrogen source among the plots (Two-way ANOVA (Year × Plot) followed by Tukey test, $p < 0.10$). C, control; I, immature compost; M, mature compost.

3.3. Bulk Nitrogen Deposition, Irrigation, Surface Drainage, Leaching, and N_2O Emission

3.3.1. Soybean Cultivation Period

The annual bulk nitrogen deposition ranged from 1.50 to 1.99 g N m^{-2} $year^{-1}$ (Figure 7, Table S6). Bulk nitrogen deposition tended to increase in the fallow season including winter. The nitrogen outputs via leaching during the cultivation season were comparable with those during the fallow season except for distinct low output in 2008–2009 (Table S7). The annual nitrogen outputs via leaching were higher in the second and third years (2009–2010 and 2010–2011, respectively: 8.51–10.86 g N m^{-2} $year^{-1}$) than those in the first year (2008–2009: 3.94–5.46 g N m^{-2} $year^{-1}$) with little leaching occurring during the cultivation season. In the first and second years the annual nitrogen leaching in both compost application plots tended to be higher than those in the control plot, but no difference among the plots was observed in the third year. Significant N_2O emissions were mainly observed in the cultivation season and after harvesting in October (Figure 7). Although the annual N_2O emissions from the immature compost plot in the first and second years tended to be higher than those from the other two plots, the difference among the plots was less clear in the third year.

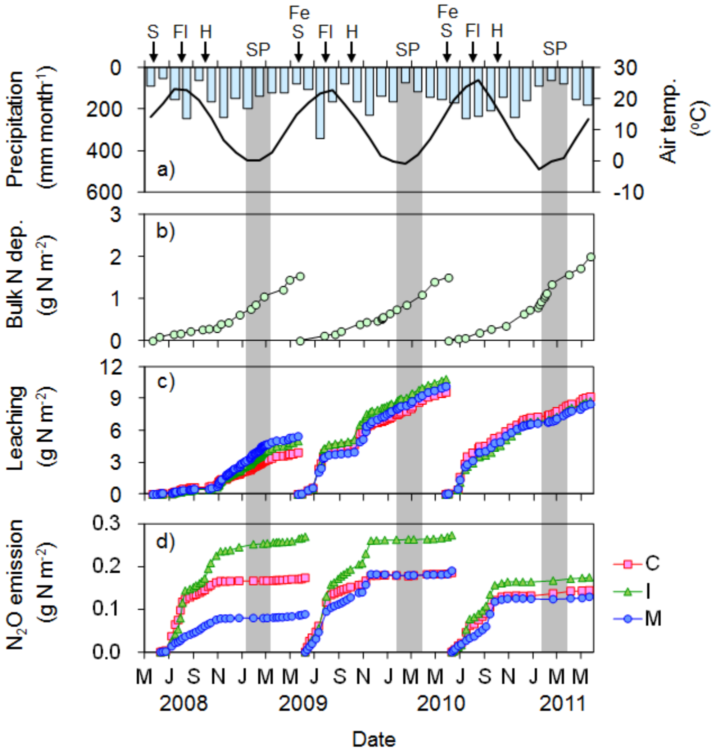

Figure 7. Seasonal changes in: monthly air temperature (line) and precipitation (bars) (**a**); bulk nitrogen deposition (**b**); leaching (**c**); and N_2O emission (**d**) during the soybean cultivation period (upland). All nitrogen flows were shown as cumulative values. C, control; Fe; Fertilizer application; Fl, flowering stage; H, harvesting; I, immature compost; M, mature compost; S, sowing; SP, snow period.

3.3.2. Rice Cultivation Period

The annual bulk nitrogen deposition in the first year (2011–2012) was lower than those in the second and third years (2012–2013 and 2013–2014, respectively; Figure 8, Table S8). The nitrogen inputs via irrigation in the second year were higher than those in the other two years; in the second year, there was an increased need for irrigation after the mid-season drainage due to low precipitation (Figure 8, Tables 1 and S8). The nitrogen outputs via surface drainage in the third year were higher than those in the other two years; the amount of water discharged during the mid-season drainage was greater in the third year due to high precipitation. There was no consistent trend in the nitrogen output via surface drainage among the plots across the three years. The nitrogen outputs via leaching during the cultivation season were lower than those during the fallow season throughout the three years (Table S9). Compared to soybean cultivation period, the nitrogen outputs via leaching in the rice cultivation period were lower during the cultivation season and comparable to that during the fallow season. The annual nitrogen output via leaching in the first year was lower than those in the second and third years due to low precipitation during the fallow season. The annual nitrogen leaching in the immature and manure compost plots tended to be higher than that in the control plot throughout the rice cultivation season. The annual N_2O emission from the immature compost plot tended to be higher than those from the other two plots throughout the three years.

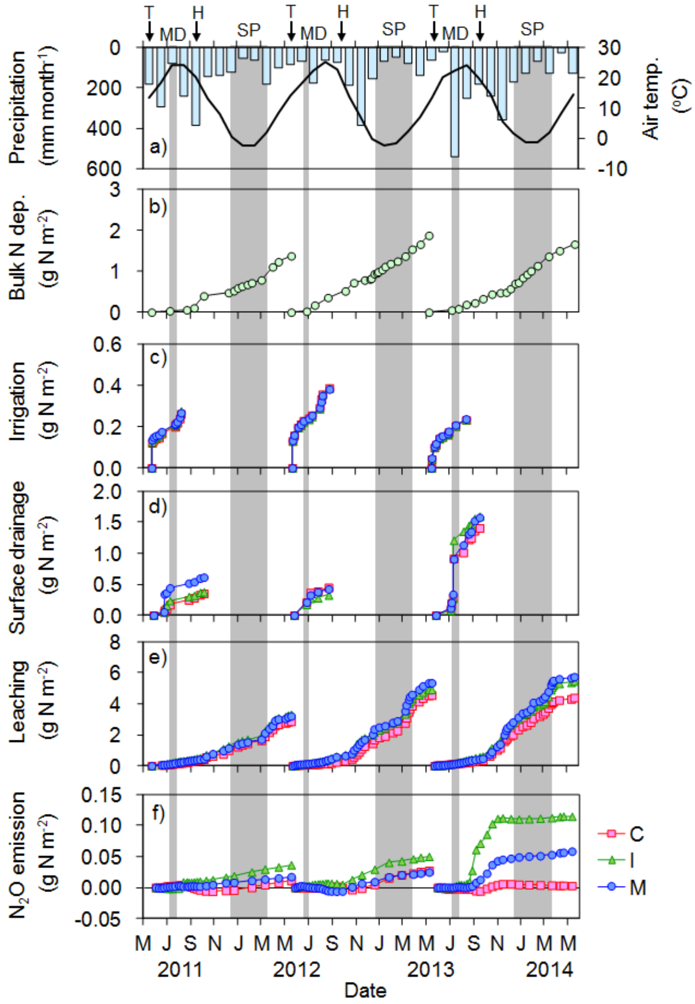

Figure 8. Seasonal changes in: monthly air temperature (line) and precipitation (bars) (**a**); bulk nitrogen deposition (**b**); irrigation (**c**); surface drainage (**d**); leaching (**e**); and N_2O emission (**f**) during the rice cultivation period (paddy). All nitrogen flows were shown as cumulative values. C, control; H, harvesting; I, immature compost; M, mature compost; MD, Mid-season drainage; SP, snow period.

3.4. Estimation of NH_3 Volatilization and N_2 Emission via Denitrification

Both NH_3 volatilization and N_2 emission via denitrification during the rice cultivation period were calculated based on the amount of nitrogen fertilizer. Therefore, their amounts increased from first year to third year with increasing in the nitrogen fertilization rate (Figure 9, Tables 2 and S8). On the other hand, their amounts were estimated to be equal among the plots irrespective of differences in physico-chemical properties of soil.

3.5. Nitrogen Budget

During the three years of soybean cultivation, the major component of total nitrogen input was symbiotic N_2 fixation (>80%), and the major components of output were harvested grain and nitrogen leaching (74%–78% and 21%–25%, respectively; Figure 9, Table S6). The total nitrogen input increased along with the difference in N_2 fixation in the following order: immature compost plot < control plot < mature compost plot, and a significant difference was found between the immature and mature compost plots. The total nitrogen output increased along with the difference in harvested grain in the following order: control plot < immature compost plot < mature compost plot, and a significant difference was found between the control and mature compost plots. During the three years of soybean cultivation under upland conditions, the annual nitrogen budgets in all plots were negative (i.e., net nitrogen loss from the field). The nitrogen loss tended to be higher in the compost application plots with higher soil nitrogen fertility than that in the control plot, although the difference was not significant.

During the three years of rice cultivation, the major component of total nitrogen input was fertilizer application (63%), whereas the major outputs were harvested grain and nitrogen leaching (48%–49% and 29%–31%, respectively; Figure 9, Table S8). Total nitrogen input varied greatly among years due to the difference in fertilizer application rates (Tables 2 and S8). The total nitrogen output increased along with differences in harvested grain and nitrogen leaching in the following order: control plot < immature compost plot < mature compost plot, and a significant difference was found between the control and mature compost plots. Both the total input and total output nitrogen flows during rice cultivation were lower than those during soybean cultivation (Figure 9, Tables S6 and S8). The amounts of nitrogen loss during the three years of rice cultivation under paddy conditions (2.3–4.3 g N m^{-2} $year^{-1}$) were lower than those during soybean cultivation under upland conditions (9.6–14.6 g N m^{-2} $year^{-1}$). The nitrogen loss tended to be higher in both compost application plots with higher soil nitrogen fertility than that in the control plot, and the difference between the control and mature compost plots was significant.

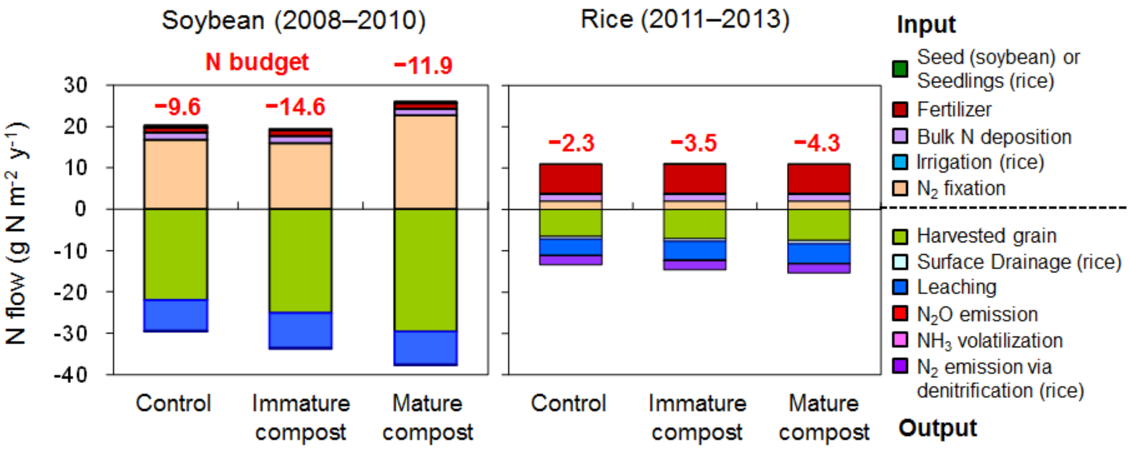

Figure 9. Effect of preceding compost application on the nitrogen (N) flows and budgets in soybean and rice cultivated field. Positive and negative values indicated nitrogen input and output, respectively. The nitrogen budget was calculated by subtracting nitrogen output from input. All values were expressed as annual value (three-year average). N_2, dinitrogen.

4. Discussion

4.1. Soil Nitrogen Fertility

Generally, loss of soil organic matter in paddy fields are considered to be lower than those in upland fields owing to slow decomposition under flooded and anaerobic condition [34,35]. In this study, however, soil nitrogen storage decreased not only during the upland soybean cultivation period but also during the flooded rice cultivation period, though the decreases during the rice cultivation period were relatively lower than those during the soybean cultivation period (Figure 2, Table S1). Soil layers that decreased their nitrogen storage were different between the periods. During the soybean cultivation period, obvious decrease in soil nitrogen storage occurred at 0–10 cm (surface soil) in both compost application plots where their nitrogen storage increased owing to preceding compost application. On the other hand, soil nitrogen storage at 0–10 cm in the control plot did not decrease during the soybean cultivation period. Nitrogen loss caused by organic matter decomposition might be compensated by nitrogen supply such as litter, harvesting residue, and rhizodeposition (e.g., [36]) from soybean and so on. During the rice cultivation period, soil nitrogen storage at 0–10 cm in all plots did not decrease or slightly increased, and the tendency agreed with previous knowledge on organic matter in flooded paddy soil [34,35]. Although the reason for decrease in soil nitrogen storage at 10–30 cm (subsoil) was not identified clearly, it might be caused by enhancement of organic matter decomposition under aerobic condition [35] owing to the presence of subsurface drainage and increase in uptake of soil nitrogen by rice due to greater root activity and expansion of the effective soil layer (i.e., root zone) to the subsoil after conversion to upland [37,38].

The inter-annual changes in available nitrogen of the surface soil roughly corresponded to the changes in their total nitrogen under the soybean-rice cultivation (Figure 3, Table S1). It was reported that the ratios of available nitrogen:total nitrogen were relatively constant among paddy soils derived from the same origin [39], while Nishida et al. [5] reported that the ratio of available nitrogen:total nitrogen of topsoils in paddy fields under paddy-upland rotation decreased along with an increase in upland frequency and increased by application of organic matter (cattle manure compost), as well as the contents of available and total nitrogen. It suggested that the easily decomposable fraction of soil nitrogen related to available nitrogen could be more sensible to upland-paddy rotation and organic matter application rather than other fractions. During the soybean cultivation period in this study, the available nitrogen:otal nitrogen ratio in the control plot did not change (Figure S1), whereas, compared to the control plot, the ratio in both compost application plots before the start of this study increased owing to preceding compost application, and then decreased throughout the soybean cultivation period, and finally decreased to the same degree as the control plot after three years soybean cultivation. Thus, manure compost application could increase available soil nitrogen through the changes in both amount and fraction of soil nitrogen, and the effect on fraction could decline by relatively short period of soybean cultivation (i.e., 3 years) after their application. The decline in available soil nitrogen after the first year of rice cultivation could be attributed to enhancement of soil nitrogen mineralization by soybean cultivation [7,40] and subsequent nitrogen uptake by rice without basal fertilization. The recovery of available soil nitrogen during rice cultivation after soybean cultivation was mainly attributed to the increase in total nitrogen, and consistent with a previous report [10].

4.2. Plant Growth, Nitrogen Accumulation, and Yield

The soybean nitrogen accumulation and grain yields in both compost application plots were higher than those in the control plot (Figures 4 and 6). The growth and yield of soybean are influenced by the nitrogen supply [6,7]. Takahashi et al. [41] also reported that soil nitrogen fertility of an upland field converted from rice paddy could be a major factor controlling soybean yield when moisture injury is not severe. During the soybean cultivation period of this study, the ground water level was relatively steady at around 60 cm [8], a suitable condition for field drainage and water supply to soybean [42]. Therefore, it is considered that the growth and yield of soybean in both compost application plots with high available soil nitrogen (Figure 3) were higher than those in the control plot. Among the plots, soybean yield in the mature compost plot was highest for three-year average.

In addition, the amount of soybean nitrogen derived from N_2 fixation was also high in the mature compost plot (Figure 4). Generally, N_2 fixation in a field with high available soil nitrogen is expected to decrease due to increased soil nitrate content via soil nitrogen mineralization [43,44]. However, the percentage of nitrogen accumulation derived from N_2 fixation in the mature compost plot was similar to that in the control plot with low available soil nitrogen (64% and 65%, respectively). This greater-than-expected fixation might be due to less suppression of N_2 fixation due to moderate supply of inorganic nitrogen by organic matter decomposition derived from mature compost [45] and direct and indirect promotion of N_2 fixation activity by high soil phosphorus availability [46–49] from the preceding mature compost application (173, 182 and 233 mg P_2O_5 kg^{-1} for the control, immature compost and mature compost plots after first year of soybean cultivation, respectively) [8].

Low 100-grain weights of soybean in 2010 (Table S4) may have been caused by high air temperature during the ripening period (mean air temperature in August was about 3 °C higher than the other two years; Table 1) [50]. The tiller numbers of rice in both compost application plots were higher than that in the control plot, reflecting their history of compost application and difference in available soil nitrogen (Tables 1 and S3, Figure 3). Although the increases in numbers of panicles and spikelets in both compost plots were partly compensated by decreases in percentage of filled spikelets, grain yields in both compost application plots tended to be higher than that in the control plot (Figure 6, Table S5). The differences in grain yield were found clearly in 2011 and 2012, years without severe damage by insects.

4.3. Nitrogen Flows

Nitrogen flows via deposition, irrigation, leaching, and surface drainage were influenced by the amount and timing of precipitation (Figures 7 and 8). High precipitation could increase deposition, leaching during the soybean cultivation and irrigation and surface drainage in the rice cultivation.

Among the measured nitrogen flows accompanied with water movement, nitrogen output by reaching was the largest. (Figure 9, Tables S6 and S8) More nitrogen was leached during the upland soybean cultivation period than during the paddy rice cultivation period, and the differences were obvious during the cultivation season (Figure 9, Tables S7 and S9). It could be due to lower organic matter decomposition (nitrogen mineralization) and nitrification in paddy soils caused by limited supply of oxygen under flooded condition during the cultivation season [35]. Nitrogen outputs via leaching in the first year of soybean cultivation (2008) were lower than those in the other two years. It might be due to low precipitation in June and July, and low soil nitrification potential in an upland field in the first year after conversion from a paddy field as compared to those in the second and third years [51].

Lysimeter experiments have a merit that nitrogen flows along with water movement can be measured precisely, whereas it has demerits (limitations) also. Because the lysimeter plots were surrounded by concrete frames, loss of flooding water via percolation through the levee (i.e., lateral seepage) did not occur. The rate of downward leaching (percolation) in this study (1 mm day^{-1}) was set lower than those in other lysimeter experiments (e.g., 3.4 mm day^{-1} in [52] and 10 mm day^{-1} in [53]). Therefore, the amount of irrigation water in this study was also less than under actual field conditions. Thus, nitrogen flows along with water movement during the rice cultivation period may have been underestimated in this study.

4.4. Nitrogen Budget

Following our previous report [8], loss of nitrogen from the soybean-cultivated upland field was quantified (Figure 9). The amount of nitrogen loss in both compost application plots tended to be higher than that in the control plot due to greater nitrogen output via harvested grain. The amounts of nitrogen loss during the soybean cultivation period estimated by the nitrogen budget agreed well with those estimated based on changes in soil nitrogen storage (Figure 10), suggesting validity of the nitrogen budget estimation in this study. Nitrogen loss during the soybean cultivation period in this study (9.6 to 14.6 g N m^{-2} $year^{-1}$) was greater than that estimated during the soybean cultivation period in a rotational field in Shiga Prefecture (3.95 g N m^{-2} $year^{-1}$) [9]. This difference might be due to the difference in soybean yield between the sites and inclusion of nitrogen leaching during the fallow season in our estimates.

Nitrogen loss during the rice cultivation period (2.3 to 4.3 g N m^{-2} $year^{-1}$) was lower than those during the soybean cultivation period (Figure 9). The N loss in this study was higher than reported previously (e.g., +1.28 g N m^{-2} in [11], +0.4 g N m^{-2} in [10]). As described above, uptake of soil nitrogen by rice in paddy fields converted from upland fields could be larger than those in unconverted paddies [31,32]. To avoid over-luxuriant growth and lodging due to the increased nitrogen uptake, nitrogen fertilization to paddy fields converted from upland fields is limited. In Akita Prefecture, it is recommended that basal nitrogen fertilization decrease by 100% and by 50%–70% in the first and second years after conversion, respectively [15]. Because the amount of nitrogen fertilization accounted for large part of nitrogen input in paddy fields (67% for three years average in this study) strongly influenced on the nitrogen budget. Therefore, nitrogen loss from the paddy fields in this study could be higher than those in previous reports and especially higher in the first year after conversion (Table S8). Unlike in the case of soybean, the nitrogen loss during the rice cultivation period estimated based on the nitrogen budget did not agree with that estimated by changes in soil nitrogen storage (Figure 10), suggesting underestimation of nitrogen loss in this study. This difference might be caused by uncertainty in the estimation of nitrogen output via NH_3 volatilization or N_2 emission via denitrification. Especially, it is well known that estimated values N_2 emission via denitrification show large variation as described by Katayanagi et al. [11]. Consequently, in the paddy–upland rotation system with rice and soybean cultivation, significant nitrogen loss may occur not only in the soybean cultivation period but also in the rice cultivation period.

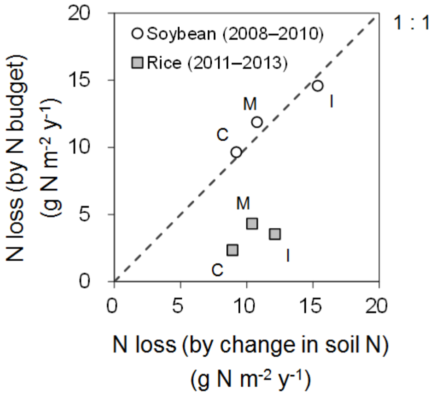

Figure 10. Comparison of nitrogen (N) loss estimated from different methods (N budget and change in soil nitrogen storage). Both nitrogen losses were converted into annual value. C, control; I, immature compost; M, mature compost.

For the six-year paddy–upland rotation in this study, the nitrogen budgets in both the soybean upland and rice paddy fields indicate net nitrogen loss from the field (Figure 9). Although the decrease in available soil nitrogen during the cultivation period was not clear, a decrease in soil nitrogen storage was observed (Figures 2 and 3). To maintain the soil nitrogen fertility in a rotated paddy field with soybean cultivation, control of the paddy–upland rotation cycle (i.e., upland frequency [5]) and application of organic matter (e.g., green manure like hairy vetch [3,54] and manure compost [5]) may be essential. However, because organic matter application to rotated paddy fields might significantly change the nitrogen flows (e.g., increase in nitrogen leaching from a paddy field [52], suppression of symbiotic N_2 fixation in soybean nodules [55]), the nitrogen budget with organic matter application should also be evaluated. On the other hand, nitrogen loss and the subsequent decrease in soil nitrogen could be an effective tool for managing soil nitrogen fertility in paddy–upland rotation in combination with organic matter application [5,10]. As observed in this study, repeated forage rice cultivation with manure compost application could excessively increase soil nitrogen fertility, making cultivation of staple rice in the field soon afterward difficult due to the risk of over-luxuriant growth. In such a case, decreasing the soil nitrogen fertility to a suitable level for staple rice cultivation by cultivating soybean could be an effective management option.

5. Conclusions

In a rotated paddy field on gray lowland soil, manure compost application during forage rice cultivation increased the growth and yields of subsequent soybean and rice crops grown in the field. Our data revealed significant nitrogen loss from the field with paddy–upland rotation, with nitrogen loss occurring during both the soybean and rice cultivation periods.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/7/5/39/s1, Table S1: Changes in soil nitrogen (N) storage in soil (0–30 cm), Table S2: Changes in nitrogen (N) accumulation in soybean and the percentage of N derived from dinitrogen (N_2) fixation during the growing period, Table S3: Changes in numbers of tillers of rice during the growing period, Table S4: Yield and yield components of soybean for three years (2008–2010), Table S5: Yield and yield components of rice for three years (2011–2013), Table S6: Annual nitrogen (N) flows and budgets during the soybean cultivation for three years (2008–2010), Table S7: Amounts of nitrogen (N) output via leaching during the soybean cultivation and fallow seasons (2008–2011), Table S8: Annual nitrogen (N) flows and budgets during the rice cultivation for three years (2011–2013), Table S9: Amounts of nitrogen (N) output via leaching during the rice cultivation and fallow seasons (2011–2014), Figure S1: Inter-annual variation of available nitrogen (N):total N ratio of the surface soil (0–10 cm) after cultivation of each year.

Acknowledgments: We are deeply grateful to the staff members of the Akita Prefectural Agricultural Experiment Station (Kazuki Sekiguchi, Keiji Sasaki, and others) for their support in the management of the experimental field. We also thank Keiko Hatakeyama, Emiko Sato, and Tomoko Suzuki for their great help with the field survey and laboratory analyses.

Author Contributions: Kazuhiro Kon and Yoshihiro Kaneta conceived and designed the experiments; Fumiaki Takakai, Takemi Kikuchi, Tomomi Sato, and Masato Takeda performed the experiments; Fumiaki Takakai analyzed the data; Kensuke Sato, Shinpei Nakagawa and Kazuhiro Kon helped with cultivation and lysimeter measurements; Fumiaki Takakai wrote the paper; and Takashi Sato and Yoshihiro Kaneta gave many constructive comments on this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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