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Nitrogen Fertilization Causes Changes in Agricultural Characteristics and Gas Emissions in Rice Field

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Abstract: Rice is a source of food for the majority of the global population. Currently, the rice yield is declining owing to extreme climate change. Farmers use nitrogen fertilizers to increase the yield; however, excessive nitrogen fertilizer application has a negative impact on plants and the environment. Nitrogen fertilizer is necessary for the growth of rice, but it is an important cause of environ-mental pollution. Carbon monoxide (CO) emitted from rice fields due to nitrogen fertilizer reacts with greenhouse gases such as carbon dioxide or methane, affecting global warming. Although CO does not directly affect global warming, it is a gas that needs attention because it reacts with various other gases. In this study, a chamber was designed and manufactured to collect the CO emitted from the paddy field after nitrogen fertilizer application in 2021 and 2022. In paddy fields, nitrogen fertilizer treatment affected the pH, EC, and soil temperature, and affected various agricultural traits. Various agricultural characteristics and the number of spikes, number of tillers, and chlorophyll content increased with nitrogen fertilizer application, whereas the amylose content decreased. Adequate nitrogen fertilizer should be applied to increase the rice yield; however, excessive nitrogen fertilizer application has a serious negative effect on grain quality and can accelerate global warming by releasing CO from paddy fields. The appropriate application of nitrogen fertilizer can have a positive effect on farmers by increasing yield. However, caution should be exercised in the application of excessive nitrogen fertilizers, as excessive nitrogen fertilizers increase the emission of CO, which affects greenhouse gases.

Keywords: grain characteristic; grain quality; CO; gas; yield

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1. Introduction

Rice is a staple food of over 60% of the world's population. Additionally, rice is used as a main energy source for the population, accounting for over 40% of the total calorie supply [1]. However, because of the recent unpredictable changes in climate, the coverage area of pest and pest occurrence is expanding. In addition, problems such as the shortening of the growth period because of temperature rise, ripening at high temperatures, yield loss, and the deterioration of grain quality occurring worldwide need immediate attention [2–4]. Farmers apply various chemicals instead of solving the problems directly, including chemical fertilizer, making this a problem that has led to an adverse impact on plant development, and environmental pollution is now being recognized as a new topic of concern [5].

When cultivating rice, farmers apply substantial amounts of fertilizer to achieve a high yield, often ignoring standard fertilization practices [6]. The appropriate application of fertilizer can help rice growth, but its excessive application is known to negatively affect not only rice growth, but also the environment [7]. Nitrogen is an essential nutrient for rice growth and metabolism, and the application of nitrogen fertilizers during the rice growing season is a common practice adopted by farmers around the world to achieve higher yields [8]. In particular, the excessive application of nitrogen causes serious damage to rice growth, yield, and grain quality, compared to non-fertilized farmlands [9]. Additionally, the nitrogen fertilizer applied to the rice fields is urea-based, which is easily volatilized into the atmosphere in the form of NH_3 [10]. Thus, farmers continue to apply more nitrogen fertilizer. However, the excessive use of nitrogen fertilizer accelerates soil pollution and global warming via residual effects [11].

Nitrogen, one of the main components of fertilizers, affects the emission of major air pollutants from the field such as carbon monoxide (CO), methane (CH_4), hydrogen sulfide (H_2S), and nitrous oxide (N_2O) [12]. Rice is sensitive to the ecological response to nitrogen, which is an essential nutrient for rice growth [13]. An adequate supply of nitrogen increases leaf area, activates photosynthesis, and is one of the factors that closely affects yield-related parameters such as an increase in dry matter through carbohydrate accumulation [14]. Nitrogen deficiency during the early stages of rice growth can reduce growth rate, inhibit chlorophyll synthesis, and cause the yellowing of leaves [15]. In contrast, an excessive supply of nitrogen increases the occurrence of diseases and pests, lodging, and the ratio of chalkiness grains to imperfect grains [16]. After the growing season, the vegetative growth is complete, new leaves and tillers are no longer generated, and root growth is gradually stopped. In the later stages of growth, nitrogen increases the protein content of grains, leading to a deterioration of grain quality [17].

Nitrogen not only influences rice growth, but also air pollutant production, global warming, and climate change [18]. The CO generated by nitrogen reacts with OH radicals in the atmosphere and changes the concentration of various air gases, such as CO_2 and CH_4, thereby greatly affecting global warming [19]. Atmospheric gases such as CO_2, CH_4, N_2O, and hydrofluorocarbon (HFCs) are directly involved in raising the Earth's temperature by absorbing the radiant heat emitted into the atmosphere, thereby causing the air effect [20]. Gases such as CO, NOx, and CO_2 are indirect air gases, as they do not directly affect global warming but can be converted into air gases by reacting with other substances [21]. Among the direct air gases, six gases, CO_2, CH_4, N_2O, HFCs, perfluorocarbons (PFCs), and SF_6, are being targeted for reduction and management by the Intergovernmental Panels on Climate Change (IPCC) to prevent global warming [22].

Rice fields are often freshwater fields. Additionally, in the rice field, soil, plants, air, and microorganisms constantly interact. These conditions catalyze the oxidation-reduction reaction of nitrogen by soil microorganisms to produce CO, CO_2, CH_4, and N_2O [23]. Though nitrogen has been applied to rice fields for a long time, since rice is very physiologically and ecologically sensitive to nitrogen, the application of nitrogen should be monitored carefully [6].

To improve the rice yield and grain quality, farmers must select cultivars with valuable genetic traits [24]. However, the soil environment, meteorological environment, and cultivation technology should also be appropriate [25]. The extent of air pollutant generation varies depending on the cultivation method. The air pollutants primarily include CO_2 from the use of agricultural machinery, NO_x from the application of nitrogen, and CH_4 from the landfilling of rice straw [26]. The amount of air gas emissions also depends on the compost application, tillage method, additional fertilizer application, and water management method of paddy fields [18,27]. The efficient use of nitrogen fertilizer can be achieved by changing the cultivation method, and the most important thing is to cultivate varieties with the highest nitrogen absorption efficiency, so that high yields can be harvested with low input [28].

Since rice is grown globally and a lot of nitrogen is used throughout the growing sea-son, there is a lot of potential for reducing air pollutant emissions by reducing fertilizer usage [29]. To reduce the gases generated in paddy fields related to greenhouse gas, re-search has been conducted to improve various cultivation methods such as water management methods, the development of new cultivars, and the use of various organic matter. However, research related to rice growth, yield, and rice quality according to nitrogen treatment is currently being presented, while research related to air pollutant emission with fertilizer treatment has been conducted by several research teams only recently. Prior to 2000, most of the studies were related to the application of nitrogen fertilizer to the rice yield [30,31]. Additionally, among these studies, a study that showed that rice yield could be increased even with nitrogen fertilizer treatment up to 100 times was included [32]. Recently, although rice yield is important, grain quality has also been recognized as a very important factor [33]. In addition, as the interest people take in climate change increases, studies on gas emissions from nitrogen fertilizer fertilization in rice fields are also increasing [18].

Therefore, this study investigated the effect of nitrogen fertilizer on agricultural traits. If nitrogen fertilizers have been treated up to 500 times in the past, this study intends to provide results for breeders by treating higher concentrations than these. Additionally, a method of directly designing and manufacturing a chamber that can collect and analyze the gases generated from rice fields was presented. Here, the gas that can be generated when nitrogen fertilizer is applied in a rice field was investigated. It was also conducted to suggest to farmers the impact that nitrogen fertilizers may have on rice yield and quality, as well as on the environment.

2. Materials and Methods

2.1. Field Design and Nitrogen Fertilizer Application

This experiment was conducted in 2021 and 2022 at the Kyungpook National University field (36°641.54″ N, 128°3826.17″ E) located in Gunwi (Agricultural Education Center, 39061, 1610, Chisanhyoryeong-ro, Hyoryeong-myeon, Gunwi, Gyeongsangbuk-do, Korea). The soil in the field was loam. Additionally, the loam property of the field is as follows: organic C 15.37 g/kg, pH 5.59, total N 2.11 g/kg. In addition, the experiment was conducted in an area where rice was continuously grown as a single crop.

2.2. Plant Material and Field Management

The gases generated during the growth of rice (*Oryza sativa* spp. *japonica* cv. Ilmi) in the paddy field were collected and investigated. Ilmi were obtained from Kyung-Min Kim at the Plant Molecular Breeding Laboratory (Kyungpook National University in Republic of Korea). Additionally, when conducting research, Ilmi was used in compliance with the international guidelines and legislation provided by the RDA (Rural Development Administration) in Republic of Korea. In addition, the rice plants were cultivated following normal local practices. This research complied with the Convention on the Trade in Endangered Species of Wild Fauna and Flora (https://www.cites.org/, accessed on 5 April 2021). The seeds were sterilized before they were sown with Spotak pesticide (25% Prochloraz, HANKOOKSAMGONG, Seoul, Republic of Korea) and soaked in darkness at 33 °C for 3 days. The sterilized seeds were sown in the field on 21 April 2021 and 25 April 2022. Thirty days after sowing, one plant per row was transplanted, and the distance between the plants was set at 30 × 15 cm. For the standard breeding of rice, N–P_2O_5–K_2O = 9–4.5–5.7 kg/10a (1000 m^2) should be applied. However, in this study, other factors were fertilized in the same way, and the rice was breeding only with different ratios of nitrogen (N). 1N means that 9 kg/10a, which is the appropriate fertilizer level, was applied during the rice growing process, and 10N, 100N, and 100N mean that 90 kg/10a, 900 kg/10a, and 9000 kg/10a were applied, respectively. Furthermore, the field was designed with 3 repetitions in each zone. Each zone was supplemented with 4.5 kg/10a of P_2O_2 (18.0% superphosphate) and 5.7 kg/10a of K_2O (62.0% potassium chloride). Additionally, the N fertilizer (46.0% urea) was provided with different time points (basal fertilizer, tillering fertilizer, and panicle fertilizer) at a ratio of 5:3:2. According to all the treatment groups, the N fertilizer was applied in a ratio of 5:3:2. The basal fertilizer was applied on 13 May 2021 and 16 May 2022, the tillering fertilizer on 13 June 2021 and 15 June 2022, and the panicle fertilizer on 20 July 2021 and 21 July 2022. A total of four treatment areas were placed, and they were cultivated according to the Agricultural Science and Technology Research Standards of the Rural Development Administration. After transplanting the rice, the water level was initially maintained at least 6 cm from the surface during cultivation. After heading, the water supply was stopped, and all the water in the field was drained. To analyze the environmental factors of the field during the air gas measurement, a temperature and humidity sensor (HMP45 C, Campbell Scientific, Inc., Logan, GA, USA) was installed 6 m from the observation tower for recording the atmospheric temperature and relative humidity. The Kyungpook National University field is 21 ha (210,000 m^2), and this study was conducted in the field closest to the temperature and humidity sensor located in the field. In addition, the nearest field and temperature and humidity sensor were located at a distance of 6 m.

2.3. Design and Fabrication of Chamber to Collect Airs in the Field

A chamber was designed to collect the air gases released during the growing period of rice in the field (Figure 1). The fabricated chamber was made of an acrylic plate made of polyvinyl chloride (PVC), with a thickness of 6 mm. The area of the upper surface of the chamber, made of polyacrylic plastic, was 615 × 455 mm. Each side of the chamber had an area of 600 mm × 1600 mm and 440 × 1600 mm. Holes with a diameter of 150 mm were made on the side and top of the chamber. The holes were left open for air circulation into the chamber when sampling was not done, and while collecting samples, the holes were closed before use. The air gas measurements were performed using a portable multi-gas detector (SKT9300DT, Test-auction, Seoul, Republic of Korea), and a lid was separately fabricated to inhale the sample during the measurement. The lid of the chamber was made to cover the upper surface of the chamber, and it was in close contact to prevent the gas in the chamber from leaking. A hose to connect the device for sample analysis was attached to the lid, and during sample collection, the hose was blocked so that the gas inside the chamber was not exposed to the outside. The air gas was collected and analyzed once every three days until harvest. In one chamber, six rice plants were transplanted at a planting distance of 30 × 15 cm (Figure 2). For the measurement of air gas, all the holes were closed to prevent the inflow of external air into the chamber or the escape of internal air, and after complete sealing for 4 h, from 9:00 am to 1:00 pm, the portable multi-gas detector was placed on the hose on the chamber lid. Since 9 am to 1 pm is similar to the daily average temperature, gas was collected from the rice field at this time. In addition, meteorological data, such as the daily average temperature and the precipitation during the course of this study, are presented on the meteorological data open portal of the Korea Meteorological Administration, and the meteorological conditions during the survey period were similar to normal years without meteorological disasters (https://data.kma.go.kr, accessed on 5 December 2022). The amount of air gases generated was investigated through linking. During gas sampling, a Takeme-10EC meter conductivity sensor (Takeme-10EC, Veinasa, Mianyang, China) was used to analyze the changes in soil anions and soil temperature with respect to the nitrogen application. Additionally, in order to simultaneously collect the gas in each chamber, researchers were located in each plot and tried to collect the gas at the same time. When collecting the gas, all the holes in the chamber were completely blocked. Furthermore, when the gas was not collected, all the holes were opened to circulate air.

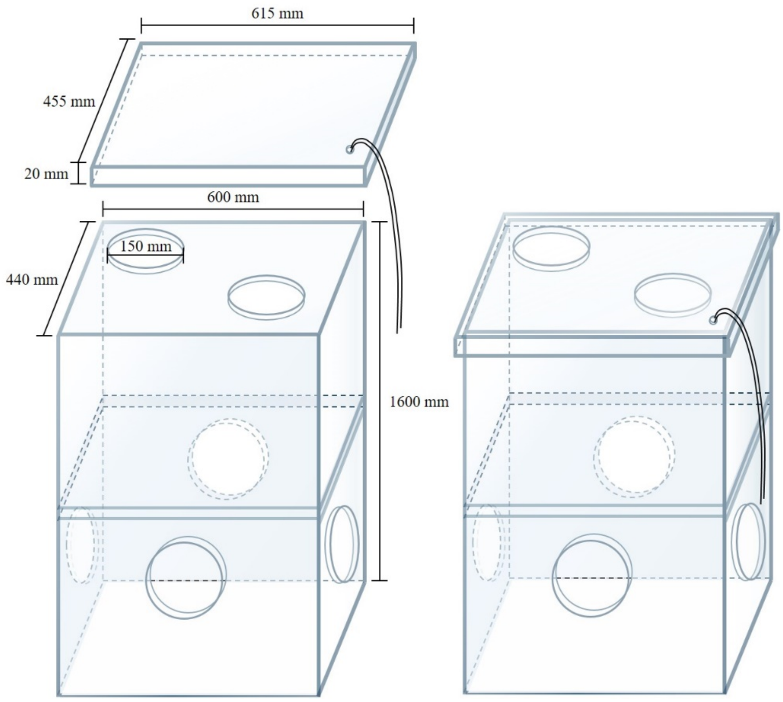

Figure 1. Diagram of the chamber designed and fabricated to collect the air gases emitted from the field. In the chamber, 8 rice plants at a distance of 30 × 15 cm were planted. One hole was made on each side of the acrylic plate and two holes on the top to facilitate air circulation in the chamber. On the upper surface, a hose for collecting air gas was connected, and while collecting air gas, all holes in the chamber were completely blocked, and the emitted air gas was collected and analyzed from 9:00 am to 1:00 pm.

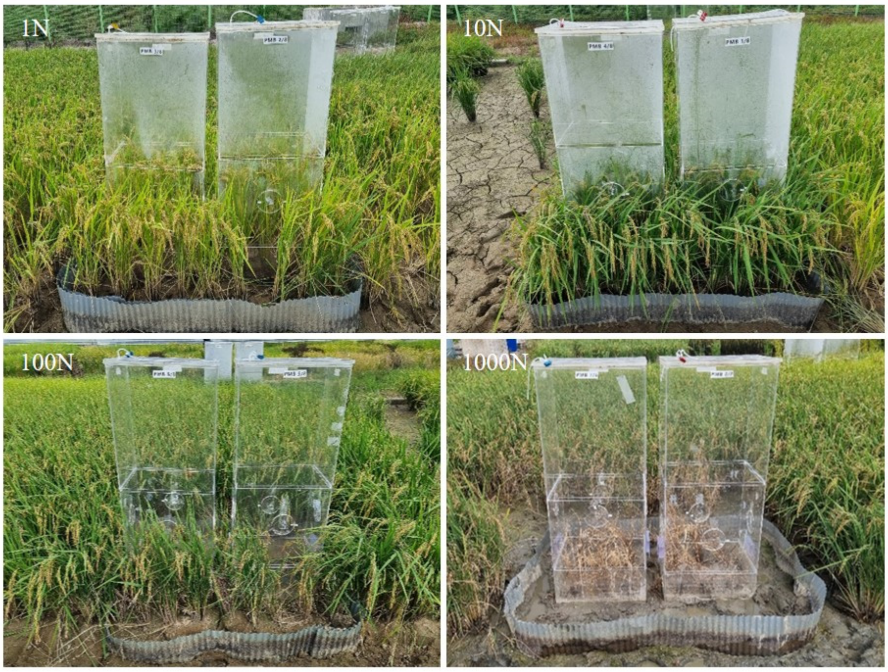

Figure 2. Air gas collection and phenotype investigation that is emitted after treatment with nitrogen fertilizer concentration using a chamber in the field. The different groups were control, 10N, 100N, and 1000N. In addition, rice traits related to yield and grain quality were analyzed under different nitrogen concentrations. It was observed that all plants survived under 10N, while some and all of the plants died under 100N and 1000N.

2.4. Soil Analysis and Major Agricultural Traits Investigation of Rice

The physicochemical properties of the soil were analyzed with respect to the different nitrogen application concentrations according to the soil chemical analysis method of the Rural Development Administration, after passing the soil sample through a 2 mm mesh after air drying. Each soil was sampled in the chamber after collecting the gas in the chamber. In addition, the soil was sampled at a depth of 10 cm in three different regions in each chamber. The soil pH was analyzed using a magnetic electrode, the Tyurin method [34], and a modified method [35], respectively. The substitution cation content was extracted with 1M NH4-Acetate (pH 7.0) and analyzed using an atomic absorption spectrometer (Analyst 800, Perkin Elmer, Whltham, MA, USA). The soil pH was measured with a pH meter after 30 min, and following the mixing and shaking of the soil with distilled water at 1:5 (w/v). The electrical conductivity (EC) was measured using an EC meter (Takeme-10EC, Veinasa, Mianyang, China) after filtering the soil with filter paper. The soil temperature was analyzed using a Takeme-10EC (Veinasa, Mianyang, China) directly in the field. In total, eight rice plants were transplanted into each chamber. The main agricultural traits that were considered were the heading date, culm length, panicle length, number of tillers, number of spikes, and chlorophyll content. The culm length, panicle length, number of tillers, number of spikes, and chlorophyll content in the field were investigated 15 days after the heading, following RDA investigation guide lines [36]. The grains were harvested 40 days after the heading [36]. After harvesting the grains, the 1000-grain weights of both the rough rice and brown rice were measured. The chalkiness grains were investigated with a visual assessment by distinguishing the endosperm with white spots, instead of being completely transparent. The chlorophyll content was investigated using a SPAD-502, and followed the manufacturer's instructions (MINOLTA, Osaka, Japan). The chlorophyll content was measured in the flag leaf at the heading date, and the main vein was avoided in the leaf and was measured in the widest region. The amylose, protein, and moisture contents were measured by a near-infrared grain tester AN-700 (Kett, Tokyo, Japan). After putting 100 g of brown rice and milled rice into the wall provided by the manufacturer, the machine was operated. After that, the machine was operated according to the instructions provided by the manufacturer.

2.5. Statistical Analysis

For the statistical analysis of the values investigated in this study, SPSS software (IMMSPSS Statistics, version 22, IBMSPSS Statistics, version 22, Redmond, WC, USA) was applied [37]. This study applied statistical analysis using survey values conducted in 2021 and 2022. A *t*-test was applied to compare and analyze the survey values of the agricultural traits according to the nitrogen fertilizer treatment in rice fields. Additionally, for an analysis of variance, one-way ANOVA was applied. When the *t*-test and one-way ANOVA analyses were performed, and the survey values were analyzed to be significant, Duncan's multiple range test (DMRT) was applied at the significance level of 5%. All experimental results in this study were obtained by independently performing three repetitions.

3. Results

3.1. Collecting and Analyzing Air Gases Emitted in the Field

When the nitrogen was first applied, O_2 and CO were emitted in all test areas in the paddy field, the amounts of which decreased over time. However, the O_2 and CO emissions, which decreased when 3 days had passed since the heading, suddenly increased and peaked when water was removed from the paddy field upon starting the heading in the field (Figure 3). In the control, the total emissions of O_2 and CO during the rice cultivation period were 304.9 $\pm$ 1.1 kg/ha and 27.2 $\pm$ 2.1 kg/ha, respectively. When the nitrogen was applied at 10N, 100N, and 1000N, the O_2 emitted from the paddy field was 308.0 $\pm$ 1.3 kg/ha, 310.7 $\pm$ 0.6 kg/ha, and 311.8 $\pm$ 0.6 kg/ha, respectively (Supplementary Figure S1). There was no difference in the O_2 emitted from the rice field depending on the nitrogen fertilizer. In addition, the total amount of CO emitted during the rice cultivation period in the paddy field treated with nitrogen at 10N, 100N, and 1000N was 38.6 ± 5.5 kg/ha, 60.5 ± 5.9 kg/ha, and 85.4 ± 3.6 kg/ha, respectively. In rice fields, the amount of CO emitted also increased when the nitrogen fertilizer was treated at high concentrations. O_2 was not affected by the application amount of the nitrogen fertilizer, but CO was released more as the amount of nitrogen fertilizer increased. After the water supply to the field was stopped in earnest when starting the heading, the emissions of O_2 and CO decreased sharply, and there were no emissions of O_2 and CO just before the harvest.

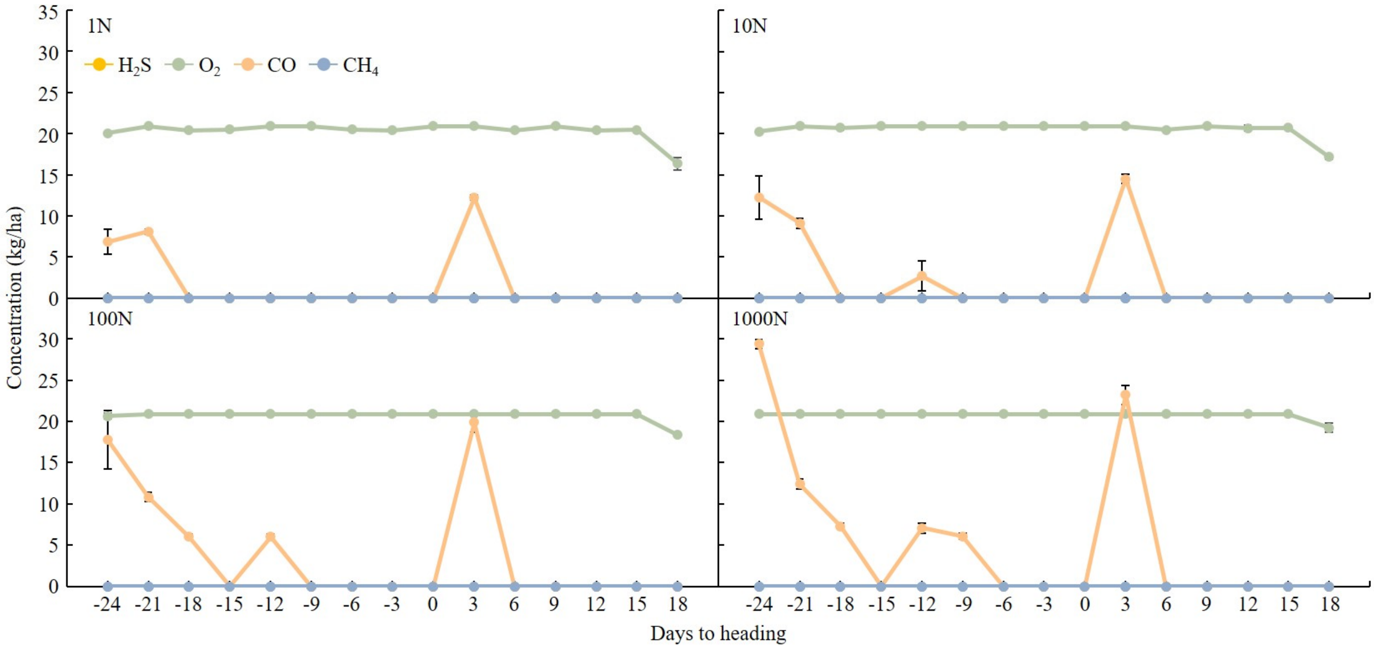

Figure 3. Emissions of air gases (H_2S, O_2, CO, and CH_4) from the field under different nitrogen concentrations (1N, 10N, 100N, and 1000N). O_2 was maintained at a constant level during the experimental period in the field. CO emission was higher at the beginning of the cultivation period, and continued to decrease and again peaked at 3 days after heading. The amount of CO emitted from the paddy field increased with nitrogen concentration. However, H_2S and CH_4 were not emitted during the experimental period. Therefore, since both H_2S and CH_4 were detected as '0', the two components overlap in the graph.

3.2. Rice Growth and Yield Characteristics with Respect to Nitrogen Fertilization Application

Figure 4 shows the results of rice growth according to the dose of nitrogen applied. When 1000N was fertilized in the field, all the rice died, and because of this, the investigation value for 1000N is not provided. The culm length of the control group was 68.4 ± 2.1 cm, which increased to 72.4 ± 2.1 (5.5% increase) cm and 73.5 ± 0.8 cm (6.9% increase) when treated with the 10N and 100N nitrogen concentration, respectively. The panicle length under the control treatment was 18.4 ± 0.9 cm, which reached 22.5 ± 0.7 cm and 21.4 ± 0.8 cm when treated with 10N (16.7% increase) and 100N (12.4% increase), respectively. The number of tillers and number of spikes were 12.1 ± 0.7 and 9.8 ± 0.9 in the control, respectively. Under 10N, the number of tillers and spikes were 15.1 ± 0.9 and 13.0 ± 1.3, respectively, which were 19.9 and 25.0% higher than the tiller and spike numbers under the control treatment, respectively. When 100N was applied, the number of tillers and number of spikes were 18.5 ± 1.1 and 16.3 ± 1.1, respectively, which were 34.7 and 40.3% higher than the tiller and spike numbers under the control treatment, respectively. The content of chlorophyll was 20.8 ± 2.1 SPAD in the control group, which increased to 45.4 ± 2.4 SPAD under 10N, and 45.8 ± 2.4 SPAD under 100N, which were 54.1 to 54.6% higher than the chlorophyll content of the control group. The number of spikelets and spikelet fertility were analyzed under control and 10N. The number of spikelets and the spikelet fertility of the group treated with 10N and 100N were compared with those of the control group. The number of spikelets were 100.3 ± 7.0, 121.1 ± 5.7, and 122.2 ± 5.810 under control 10N and 100N, respectively. Under 10N, the number of spikelets increased by 17.2% compared to the control group, while under 100N, it increased by 17.9%. The spikelet fertility was 93.0 ± 2.4% in the control group, which increased to 93.5 ± 1.6% (a 0.46% increase compared to under control) and 92.5 ± 1.6% (0.59% lower than under control) when the nitrogen was applied at 10N and 100N, respectively.

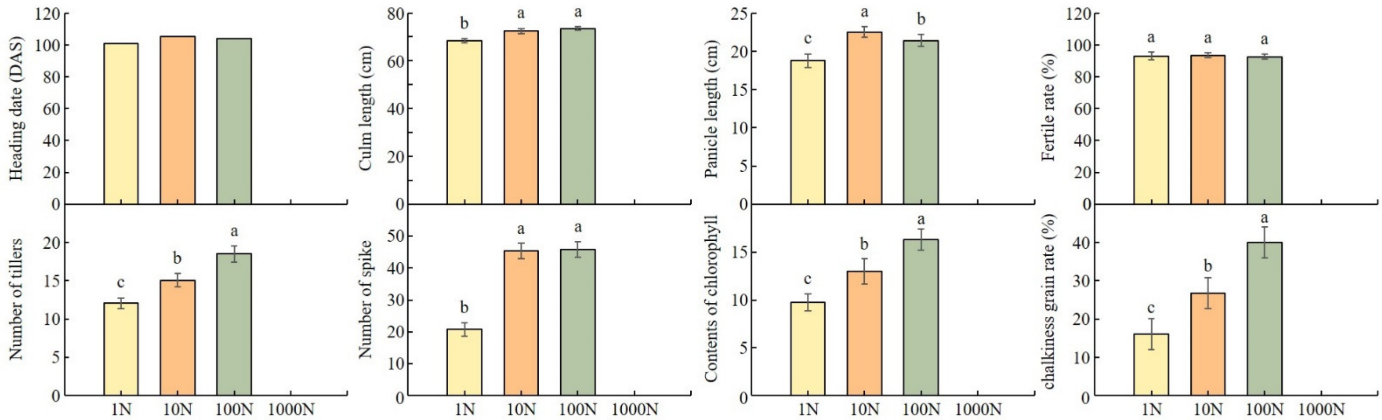

Figure 4. Yield-related traits of rice under various application doses of nitrogen (1N, 10N, 100N, and 1000N). All plants died at 1000N fertilization. Most of the agricultural traits, except for fertility rate, increased with increase in nitrogen dose. Data are expressed as the mean ± SD from five independent samples per treatment. Bars represent means ± standard error. Means denoted by the same letter are not significantly different ($p < 0.05$) as evaluated by Duncan's multiple range test.

3.3. Grain Characteristic with Respect to Nitrogen Fertilization Application

The seed characteristics were analyzed according to the dose of nitrogen (Figure 5). The 1000-grain weight; grain length; grain width; grain thickness; and ratio of grain length/width were investigated separately for rough rice and brown rice. When the nitrogen was applied at 1000N, the plants died, and no seeds were harvested. Therefore, the investigation value when 1000N was processed was not provided. In the control group, the 1000-grain weights of the rough rice and brown rice were 21.8 ± 0.9 g and 19.5 ± 1.4 g, respectively, and the 1000-grain weights of the rough rice and brown rice when treated with 10N were 25.3 ± 1.6 g (13.6% increase) and 21.3 ± 0.9 g (8.8% increase), respectively. Under 100N, the 1000-grain weights of the rough and brown rice were 26.5 ± 1.5 g and 22.3 ± 0.9 g, respectively, which were 4.6% and 9.2% higher than those of the control group of the rough rice and brown rice, respectively. In the control group, the grain lengths of the rough and brown rice were 7.2 ± 0.1 mm and 4.9 ± 0.2 mm, respectively. However, when 10N was applied, the grain lengths of the rough rice and brown rice were 7.4 ± 0.2 mm and 5.3 ± 0.1 mm, respectively, achieving an increase of 3.1% and 7.6% for the rough rice and brown rice, respectively. When the nitrogen was applied at a 100N, the grain lengths of the rough rice and brown rice were 7.6 ± 0.2 mm and 5.3 ± 0.1 mm, respectively, and compared to that of the control group, the grain length increased by 4.6% and 9.2% in the rough rice and brown rice, respectively. The grain width of the rough rice was 3.3 ± 0.1 mm; 3.6 ± 0.1 mm; and 3.7 ± 0.1 mm, respectively, under the control, 10N, and 100N, respectively. The grain width of the rough rice increased by 7.9% and 8.9%, respectively, compared to that of the control group. Furthermore, the grain width of the brown rice was 2.9 ± 0.1 mm; 3.1 ± 0.1 mm; and 3.2 ± 0.1 mm in the control, 10N, and 100N, respectively, which decreased by 66% and 93% compared to that of the control. The grain thickness of the rough rice was 2.3 ± 0.1 mm; 2.6 ± 0.1 mm; and 2.6 ± 0.1 mm, and that of the brown rice was 1.9 ± 0.2 mm; 2.1 ± 0.1 mm; and 2.2 ± 0.1 mm when treated with the control, 10N, and 100N, respectively. When the nitrogen was applied at 10N and 100N, the grain thickness decreased by 10.9% and 10.6% in the rough rice, and by 7.1% and 9.8% in the brown rice, respectively. In the control group, the ratio of the grain length to the width of the rough rice was 2.2 ± 0.1 in the control group and 2.1 ± 0.1 (5.3% lower than the control value) when the nitrogen was applied at 10N. When the nitrogen was applied at 100N, the ratio of the grain length to the width was 2.1 ± 0.1 (4.8% lower than the control value). In addition, the ratio of the grain length to the width of the brown rice was 1.7 ± 0.1; 1.7 ± 0.1; and 1.7 ± 0.1 in the control, 10N, and 100N groups, respectively. Although the width increased by 0.9% compared to that of the control group, the ratio of the grain length to the width of the brown rice decreased by 0.2% when the nitrogen was applied at 100N.

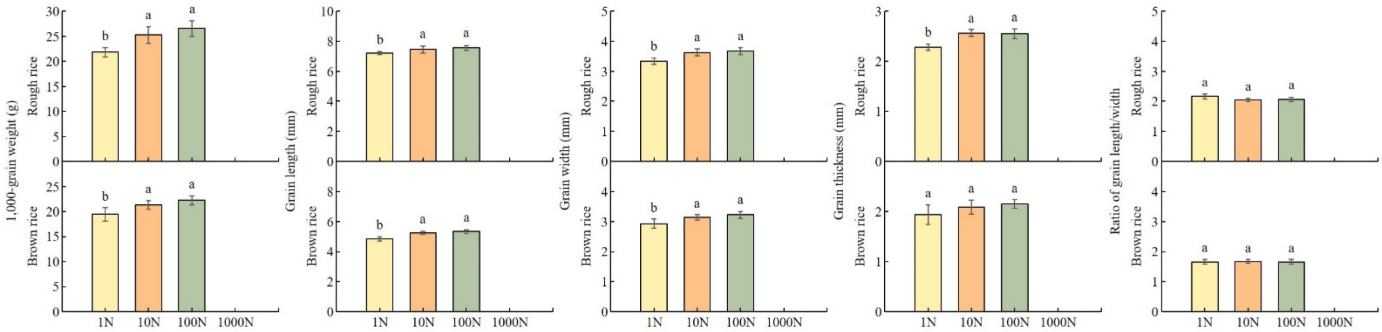

Figure 5. Changes in rice grain traits under different nitrogen concentrations. Under control, 10N, and 100N, the grain thickness and ratio of grain length/width of the grains were same. However, the yield affecting factors such as 1000-grain weight, grain length, and grain width increased after nitrogen application compared to those of the control. Under 1000N, all plants died. Data are expressed as the mean ± SD from five independent samples per treatment. Bars represent means ± standard error. Means denoted by the same letter are not significantly different ($p < 0.05$) as evaluated by Duncan's multiple range test.

3.4. Changes in the Grain Quality with Respect to Nitrogen Application

The contents of the protein, amylose, and moisture, which are the factors related to the grain quality, were compared after the nitrogen application at 10N, 100N, and 1000N (Figure 6). When the nitrogen was applied at a 1000N, the plants died and the seeds could not be harvested. In addition, the grain quality of the brown rice and milled rice was investigated separately. In the control group, the protein, amylose, and moisture contents of the brown rice were 6.0 ± 0.3%, 17.6 ± 0.2%, and 14.5 ± 0.3%, respectively, while these were 6.6 ± 0.1%, 15.7 ± 0.7%, and 14.3 ± 0.2% when applied at 10N, respectively. The contents of the protein, amylose, and moisture were 6.9 ± 0.1%, 15.3 ± 1.6%, and 14.7 ± 0.3%, respectively, when nitrogen was applied at a 100N. The protein content in the brown rice increased by 9% and 12.6% when the nitrogen was applied at 10N and 100N, respectively, but decreased by 11.7% and 15.1% for the amylose content, respectively. Although it decreased by 1.6%, the water content increased by 0.9% when the nitrogen was applied at 100N. The protein, amylose, and moisture content of the milled rice in the control group were 6.1 ± 0.1%, 17.3 ± 0.2%, and 14.5 ± 0.2%, respectively, and when the nitrogen was applied at 10N, these were 6.6 ± 0.1%, 15.2 ± 0.8%, and 14.5 ± 0.2%, respectively, and when applied at 100N, these were 6.8%, 15.2%, and 14.2%, respectively. When treated with 10N, the protein content and water content increased by 7.1% and 0.2%, respectively, but the amylose content decreased by 14.0%. In addition, when the nitrogen was applied at 100N, the protein content increased by 10.2%, but the amylose and water contents decreased by 14.0% and 2.1%, respectively.

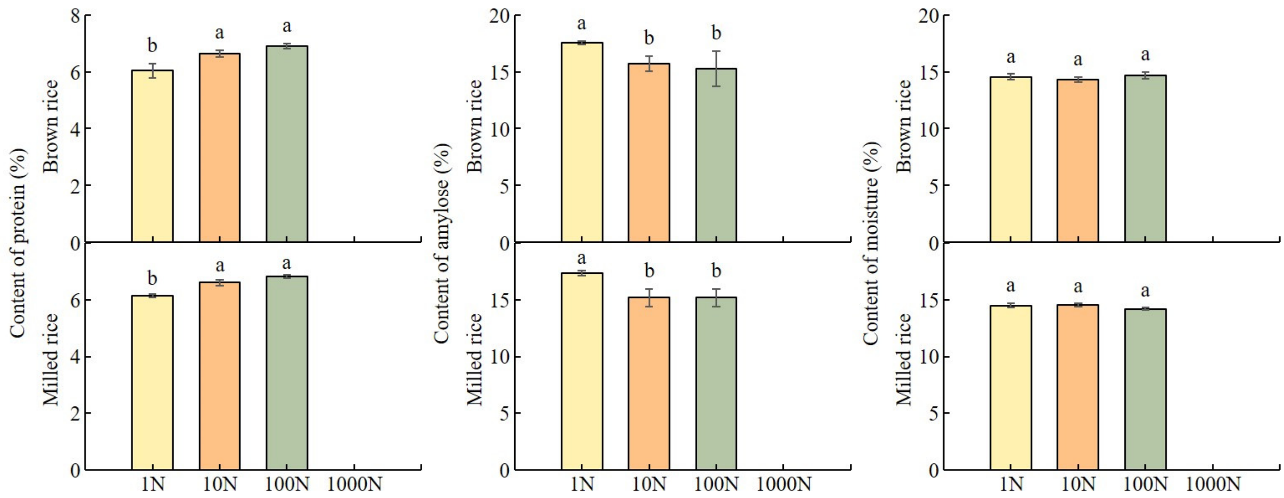

Figure 6. Comparison of factors related to grain quality of brown rice and milled rice under different nitrogen concentrations. Brown rice and milled rice were investigated for protein, amylose, and moisture content in grains under control, 10N, and 100N of nitrogen concentration. In both brown rice and milled rice, higher nitrogen application increased the protein content and lowered the amylose content, which affected the grain quality, with no effect on moisture content. Data are expressed as the mean ± SD from five independent samples per treatment. Bars represent means ± standard error. Means denoted by the same letter are not significantly different ($p < 0.05$) as evaluated by Duncan's multiple range test.

3.5. Change of Field pH and Redox Potential (Ec) According to Nitrogen Application Amount

As shown in Figure 7, the soil pH was 6.0–6.2 during the cultivation period in the control group, but under the nitrogen applied at 10N, 100N, and 1000N, the soil pH reached 5.5–5.7, 5.3–5.5, and 5.0–5.3, respectively. The pH of the rice cultivation field decreased with the increased nitrogen dose. The EC of the soil was 0.4–0.2 in the control group, and 0.2–0.7, 0.3–0.9, and 0.3–1.0, when 10N, 100N, and 1000N were applied, respectively.

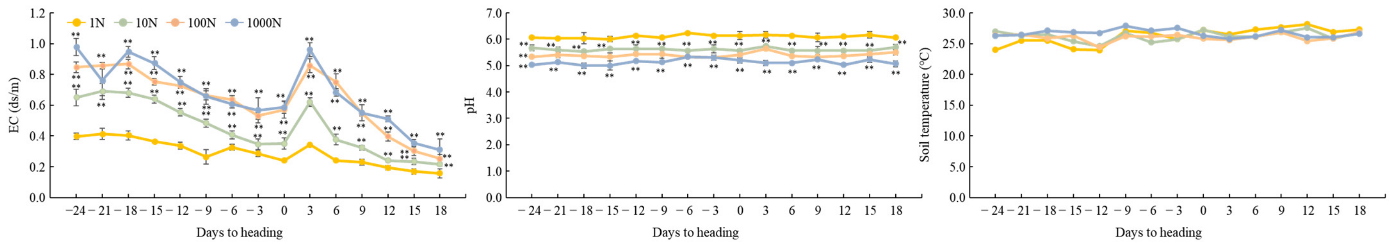

Figure 7. Changes in electric conductivity (EC), pH, and soil temperature with respect to nitrogen dose during rice cultivation. EC value decreased with time after nitrogen application, but peaked after heading in field, when water was removed from the paddy field, and continued to decrease thereafter. With increase in nitrogen application, the pH was closer to neutral, and no significant difference in soil temperature was observed. ** mean significant difference at $p < 0.01$ by *t*-test.

3.6. Agricultural Traits of Rice Most Sensitive to Nitrogen

The most sensitive parameters of rice, related to botanical and grain characteristics, and grain quality among each agricultural trait, were analyzed under control, 10N, 100N, and 1000N (Figure 8). The most sensitive reaction to the application rate of the nitrogen was analyzed by creating a heat map in which the change in trait characteristics was large. As the concentration of the nitrogen increased, the number of tillers, number of spikes, and chlorophyll content increased, and the degree of increase was considerable for each parameter. However, as the dose of nitrogen increased, the amylose content decreased in both the brown and milled rice. Other investigated traits were also affected by the nitrogen, though were not significant.

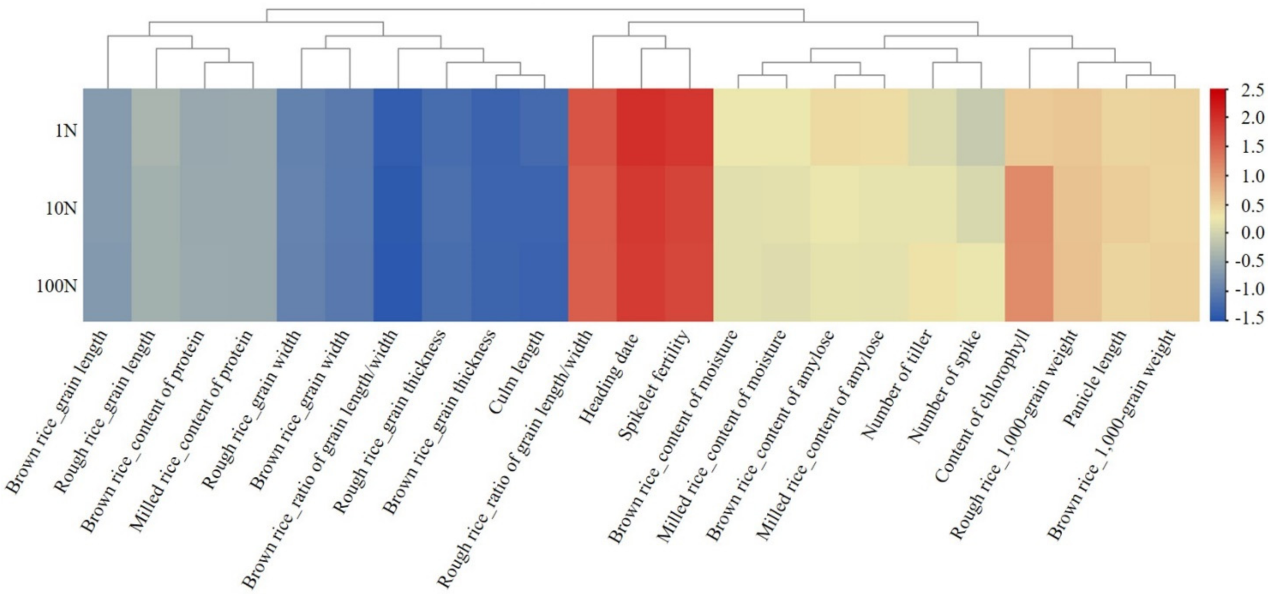

Figure 8. Changes in most sensitive rice traits under different nitrogen fertilizer concentrations. The red and blue levels of the heat map mean a high and low concentration of nitrogen, respectively. Number of spikes, number of tillers, and chlorophyll content increased, while amylose content decreased as the dose of nitrogen increased.

4. Discussion

In this study, all of the major agricultural traits investigated showed a general tendency to decrease with an increased nitrogen concentration, and this showed the same trend as the previously reported effect of nitrogen on yield [38]. In the case of grain, the protein content increased and the amylose content decreased with an increase in the nitrogen concentration, which was consistent with previous studies [39]. Therefore, the nitrogen treatment had a direct negative effect on the grain quality. In general, it has been reported that japonica cultivars with a good taste have a low protein content and a high amylose content, and thus have a negative effect on the grain quality from excessive nitrogen [40,41]. In this study, the higher the application of nitrogen, the higher the chalkiness in grains, negatively affecting the commercial value and grain quality [40]. In particular, chalkiness grains temporarily produce grains with poor maturation because of an imbalance in the supply of substances within the grain during the ripening period [42].

Additionally, the content of chlorophyll is related to the accumulation of anabolic products, and ultimately has an effect on yield [43]. The content of chlorophyll increased when the nitrogen treatment in this study was increased. However, although the increase in the chlorophyll content had a positive effect on the yield, as a result, the protein content of the grain was increased, which had a negative effect on the grain quality [44]. Therefore, it can be said that it is important to treat nitrogen fertilizer at an appropriate level to have a positive effect on yield and not negatively affect grain quality. In addition, in this study, the pH in the soil was analyzed when the nitrogen fertilizer was added. Methanogens in the soil are obligate anaerobic bacteria and show their maximum activity around pH 7 [45]. However, in this study, the higher the nitrogen fertilizer treatment, the lower the soil pH. As a result, the activity of the methane-producing bacteria decreased, so it is thought that methane was not detected in this study. In particular, methane-producing bacteria are highly active in fields containing a lot of organic matter, such as rice straw, and a large amount of methane is generated [46]. However, it is considered that rice straw and organic matter are contained at a low level in the rice field of this study.

Several studies have already reported that nitrogen fertilizer treatment in rice fields can stimulate the emission of various greenhouse gases [47,48]. In addition, in this study, it was shown that the increase in nitrogen fertilizer also increased the emission of CO in the rice field, and it was increasing in the same trend as the previous study. It was reported that the use of fossil energy increased rapidly because of its rapid industrialization, and the concentration of CO in the atmosphere rapidly increased, accelerating global warming [49]. Recently, rice paddy fields that supply food to the majority of the global population have accounted for a large portion of the world's CO emissions [50,51]. In particular, the main cause of CO emissions from rice paddy fields is the application of excessive nitrogen that also affects plants and contributes to global warming [50]. Nitrogen fertilizer treatment converts rice paddy fields into anaerobic agricultural soil, increasing the emission of greenhouse gases such as CO [52]. Overall, the emission of CO was significantly greater with the higher treatment content of the nitrogen fertilizer, as compared to the control group. This is a common reaction, such as the oxidation of soil organic matter with nitrogen fertilizers [53]. As a result, more carbon substrates also acted excessively to increase the rice growth and yield [53].Furthermore, immediately after the rice transplantation into the field, excess CO was generated during the de-composition of the organic matter in the soil, and was directly emitted from the soil into the air [54]. However, as plant growth progresses, the emission of CO also increases, owing to the development of aeration tissue. More than 90% of the CO emissions during the rice cultivation period are generated by the rice aeration system, and only the remaining 10% is emitted from the soil by simple diffusion [55]. In this study, when the nitrogen was applied, a positive effect was observed on the culm length and most plant characteristics, and it had a significant effect on the CO emissions. Therefore, it has been reported that there is a positive correlation between CO emissions and altitude when the growth-related factors of the aboveground part of rice are tied [56], which is consistent with the results of the present study. In this study, CO emissions were particularly high, but the amount and type of CO emitted from the field may vary because of the differences in the materials and environmental factors prevailing in the study [57]. In addition, the increase in CO emissions during the initial period of the rice growing period was obvious, because the nitrogen applied in freshwater was decomposed under anaerobic conditions, and the organic carbon content was greatly increased [58].

In paddy fields, CO is emitted by various factors including fertilizer treatment [59]. There are cultivars that emit CO easily, owing to the genetic makeup of plants, but the temperature and precipitation during rice cultivation have a great influence on CO emissions. Since the gas exchange between the soil and the atmosphere is facilitated by soil microorganisms, it is one of the most important influencing factors for the CO emission from paddy fields [60]. In addition, rice is cultivated in paddy fields, which are in fresh water for most of the growing period, from sowing to harvest. In freshwater conditions, anaerobic conditions are maintained, and the organic matter in the soil is decomposed by microorganisms such as methanogens [29], with the gas finally emitted through the rice aeration system [61]. Therefore, simply reducing the concentration of nitrogen is not a complete solution for reducing the CO emissions from paddy fields. Currently, various cultivation methods are being studied to reduce or replace nitrogen fertilizer. It has been reported that increasing the phosphorus content can maintain the rice yield and reduce the amount of greenhouse gases emitted from rice fields [61].

Nitrogen is related to rice yield. Additionally, this is not simply related to agricultural traits, but to the various gases that can change the composition of the atmosphere. Proper levels of nitrogen fertilizer can benefit farmers by increasing their yields. Excess nitrogen, however, negatively affects the grain quality and releases greenhouse gas-related gases from rice fields. Therefore, additional in-depth studies are needed to determine the appropriate concentration level of nitrogen fertilizer that can bring the maximum benefits to farmers while minimizing the negative impacts on the environment.

5. Conclusions

The effect of nitrogen application to paddy fields on the emissions of soil, rice plant, and CO was investigated. Furthermore, a chamber to collect the CO emitted from the paddy field was designed directly. Nitrogen fertilizer treatment not only has a positive effect on rice growth in a rice field, but may also have a negative effect. In addition, excessive nitrogen fertilizer releases various gases related to greenhouse gases in rice fields. In this study, only CO and O_2 were released from the rice field, which was a factor greatly affected by the soil conditions. The soil investigated in this study contained relatively little organic matter, and methane was released from the rice field when the soil contained a large amount of organic matter. Therefore, rather than unconditionally reducing nitrogen fertilizer, it is important to analyze the soil composition before applying fertilizer and apply the nitrogen fertilizer accordingly. It is suggested that the application of appropriate fertilizers through a soil composition analysis can slow down global warming while yielding a high yield and quality rice. Additionally, it is necessary to establish an appropriate nitrogen treatment concentration in the rice growth process and to breed rice cultivars that can effectively absorb the nitrogen fertilizer. In this study, basic data for this were provided.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15043336/s1, Figure S1: total greenhouse gas emissions during the irradiation period in the paddy field. The greenhouse gas emissions after treatment with nitrogen fertilizer 0N, 10N, 100N, and 1000N in the paddy field were investigated. Emissions of H_2S, O_2, CO, and CH_4 were investigated, but H_2S and CH_4 were not detected. However, O_2 and CO were detected in the paddy field. O_2 was not affected by nitrogen fertilizer, and even if the concentration of nitrogen fertilizer increased, the amount of O_2 emitted from the paddy field was the same. However, CO emission was different according to the effect of nitro-gen fertilizer, and it was 27.2 ± 2.1 in the control, but it was 38.6 ± 5.5, 60.5 ± 5.9, and 85.4 ± 3.6 when the concentration of nitrogen fertilizer was 10 times, 100 times, and 1000 times, respectively. As the concentration of nitrogen fertilizer increased, the amount of CO released from the paddy field also increased.

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