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Influence of Nitrogen Limitation and Long-Term Use of Rockwool on Nitrous Oxide Emissions in Hydroponic Systems

Shin-nosuke Hashida[1*], Kazuyoshi Kitazaki[2], Kazuhiro Shoji[1], Fumiyuki Goto[1] and Toshihiro Yoshihara[1]

[1]*Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), 1646 Abiko, Chiba 270-1194, Japan*

[2]*National Agriculture and Food Research Organization (NARO), Kyushu Okinawa Agricultural Research Center, Japan*

[*]**Corresponding author:** Shin-nosuke Hashida, PhD, Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), 1646 Abiko, Chiba 270-1194, Japan, Tel: +81-4-7182-1181; E-mail: shashida@criepi.denken.or.jp

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Abstract

To mitigate Nitrous Oxide (N_2O) emissions derived from Nitrogen (N) fertilizer of agroecosystems, establishment of best management protocols for cultivation is necessary. Hydroponic systems using rockwool have the potential to reduce N_2O emissions; however, the effects of nutrient condition and retained N compounds in rockwool on N_2O emissions remain unclear. The primary objective of our study was to understand the crucial factors behind emissions of N_2O. Tomato cultivation with low levels of nutrient showed reduced growth and yield, but increased N_2O emission. In contrast, growth and N_2O emissions were increased by cultivation with normal levels of nutrient and used (1-y-old) rockwool containing excess N compounds from the previous year's cultivation. Though the long-term use of rockwool significantly enhanced seasonal N_2O emission, the availability of N_2O precursors NO_3^- and NH_4^+ did not clearly explain the variation in N_2O fluxes during cultivation. Rather, environmental factors, such as relative water content of rockwool in the rhizosphere, were significantly correlated to N_2O emissions during cultivation under various conditions. We conclude that environmental factors most strongly influence the fate of available environmental substrates remaining in rockwool, and thereby control N_2O emissions.

Keywords: Denitrification; Greenhouse Gases (Ghgs); Nitrous Oxide (N_2O); Rockwool; Tomato

Introduction

Increasing atmospheric Nitrous Oxide (N_2O) is a major concern in agriculture because of its high Global Warming Potential (GWP) as a Greenhouse Gas (GHG); the GWP for a 100-year timescale of N_2O is 310 times higher than that of Carbon Dioxide (CO_2) [1]. In addition to its global warming potential, N_2O is currently the largest ozone-depleting substance in the atmosphere and is projected to maintain that status throughout the 21st century [2]. It is recognized that agriculture is responsible for two-thirds of global N_2O emissions, and that N_2O formation is a result of excessive Nitrogen (N) fertilization [3]. Feasible ways to mitigate N_2O emissions in agricultural production include improving nutrient use efficiency of crops, improving land-management and cultivation practices, and abandonment of cultivation [4]. Inorganic N compounds in soil, such as Nitrate (NO^{3-}) and Ammonium (NH_4^+), that are not taken up by plants can be processed through the bacterial respiratory pathways of nitrification and/or denitrification, and converted to N_2O [5-7]. A number of studies have reported the significant role of microbial respiration in N_2O production in soil [8,9]. However, soil environmental factors, in addition to size and composition of the microbial community, also influence N_2O fluxes [10,11].

Currently, the plant and horticultural industries are focused on stable production, quality improvement, and high yield. An increasing number of studies focusing on the environmental impacts of horticultural practices have reported that GHG emissions from horticultural production arise mainly from cultivation processes rather than from industrial production of materials used in cultivation (e.g., electricity, fertilizer, biocides, and rockwool) [12-15]. N_2O emissions and total nitrogen losses from rockwool systems are predicted to be caused primarily by microbial denitrification or chemodenitrification [16,17]. However, the extent to which changes in N_2O emissions are explained by changes in management practices during the cultivation process remains elusive. In our previous work, hydroponic cultivation using rockwool produced substantial tomato fruit yield while lowering CO_2 emissions during the growing season, by suppressing microbial proliferation in the rhizosphere [18]. On the other hand, conventional hydroponics did not effectively mitigate N_2O emissions. Consequently, emission of N_2O was governed by neither the size nor the composition of the microbial community. Thus, as for soil-based cultivation [19], abiotic stimulation of rhizobacteria is important to N_2O emissions from rockwool systems, although microbial proliferation is also essential for N_2O production. Environmental factors can influence microbial and chemodenitrification [17], but the effects of nutrient dynamics, such as macro- and microelements, on N_2O fluxes in the rhizosphere during crop cultivation remain unclear. A predictable factor for microbial stimulation in the rhizosphere is the concentration and form of inorganic N compounds. Enzymatic activity and availability of substrates for denitrification could act as determinants for N_2O production. In addition to the concentration and form of inorganic N compounds, other elements are likely to affect the absorption of NO^{3-} and NH_4^+ by plants, and thus influence the nutritional status of the rhizosphere. Understanding the precise mechanisms of N_2O production in rockwool systems is important to controlling N_2O emissions from these systems.

In this study, we measured GHG emissions and nutrient dynamics using hydroponics with a rockwool system. Our objective was to examine the effect of long-term use of rockwool, nutrient concentration, and the interaction between these factors on N_2O flux during hydroponic cultivation. In addition, we explored the factors that are relevant to N_2O emissions to better understand management practices for mitigating N_2O emissions.

Materials and Methods

Plant material and growth conditions

Tomato (*Solanum lycopersicum*, Momotaro) plants were hydroponically grown with rockwool in an air-conditioned greenhouse in Abiko, Chiba, Japan (35.9°N, 140.0°E) from April to September 2012. The average temperature was set at 23 ± 3°C. Each three-week-old tomato seedling grown in rockwool block (Grotop Master, Gordan, Denmark) was transplanted to a 1/2000 a (12 L) Wagner pots filled with freshly prepared or once-used rockwool blocks sub-irrigated with a nutrient solution of the following composition (mg L^{-1}) 179 N-NO^{3-}, 18 N-NH_4^+, 92 P, 312 K, 177 Ca, 46 Mg, 2.1 Fe, 1.2 Mn and B, 0.07 Zn, 0.02 Cu and Mo (Otsuka House Solution A, Otsuka Chemical Co. Ltd., Osaka, Japan) with Electrical Conductivity (EC) of 1.0 dS m^{-1} (EC1.0) or 0.5 dS m^{-1} (EC0.5). During cultivation, the nutrient solution was supplied using 100 mL h^{-1} drip irrigation. Cultivation with freshly prepared rockwool and EC0.5 is malnutrition condition while cultivation with once-used rockwool and EC1.0 seems to be over-nutrition condition. Fruits were harvested every 2 wk from July to September, and total fresh weight per pot (fruit yield) and Brix value of individual harvested fruit were measured. Each plant height was measured at the end of cultivation. Old leaves and lateral shoots were removed on a weekly basis, dried, and weighed to estimate total shoot biomass. The investigations were performed with 3 replicates.

Experimental setup and gas sampling

We established four experimental blocks: Freshly Prepared Rockwool (FR) supplemented with nutrient solution at EC1.0 (FR-EC1.0) or EC0.5 (FR-EC0.5), and rockwool used for 1 y as FR-EC1.0 (UR) with nutrient solution at EC1.0 (UR-EC1.0) or EC0.5 (UR-EC0.5). CO_2, CH_4, and N_2O emissions from the pots were captured using a closed-chamber technique as described before [18].

Measurements of GHG fluxes and ionic components

A gas-chromatography 7890A GC system equipped with a HaySepQ80/100 separation column (Agilent Tech. Inc., Santa Clara, CA, USA) was used to measure CO_2, CH_4, and N_2O as described before [18]. Ionic components, Cl^-, NO_3^-, PO_4^-, SO_4^{2-}, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}, were extracted from rockwool of the rhizosphere with a homogenizer (Retsch MM300, Haan, Germany), followed by distilled water extraction, and measured with an ICS-1500 ion chromatography system (Dionex Corp., Osaka, Japan). Accuracy was established using cation mixed standard solution II and anion mixed standard IV (Kanto Chemical Co., Inc., Tokyo, Japan).

Statistical analysis

Two-way analysis of variance (ANOVA) with repeated measures was used to detect significant effects of rockwool type, nutrient concentration, and their interaction, using Excel 2008 Statistics for Windows (Microsoft Corporation, Redmond, WA, USA). Differences in growing-season plant growth, fruit yield, and N_2O emissions among the four treatments were analyzed by Dunnette's test using KyPlot 4.0 (KyensLab Incorporated, Tokyo, Japan). Correlations between N_2O emissions, dynamics of nutrient concentrations, relative water content (RWC) were analyzed by Spearman's rank correlation test using KyPlot 4.0.

Results and Discussion

Plant growth and fruit yield in conditions of over-nutrition and malnutrition

Shoot biomass at the end of the cultivation period was significantly higher in used rockwool (UR) treatments than in freshly prepared rockwool (FR) treatments regardless of nutrient concentration (Table 1).

		FR		**UR**	
		EC 1.0	**EC 0.5**	**EC 1.0**	**EC 0.5**
Body	Height (cm)	212 ± 8.3	151.8 ± 29.9	168.3 ± 15.4	148.5 ± 37.5
	gDW	95.2 ± 4.5	*42.8 ± 5.8*	**131.6 ± 16.5**	82.4 ± 5.3
	No. fruit branch	6.3 ± 0.5	*4.7 ± 0.5*	6.7 ± 1.2	*4.3 ± 0.5*
Litter	gDW	2.3 ± 1.2	2.3 ± 1.4	2.7 ± 0.5	1.4 ± 0.1
Total	gDW	97.5 ± 4.7	*45.1 ± 5.6*	**134.3 ± 16.3**	83.8 ± 5.3
Fruit	Number	14 ± 4.5	11.7 ± 1.7	15.3 ± 3.9	15.3 ± 0.9
	Yield (gFW)	1209.1 ± 156.1	873.5 ± 130.6	987.6 ± 227.8	1028.7 ± 71.5
	Averaged size (gFW)	62.5 ± 49.2	69 ± 37.2	50.2 ± 36.9	61 ± 30.3
	Averaged Brix	6.4 ± 0.8	5.5 ± 0.7	7.2 ± 1	6.4 ± 1.2

	Brix yield	7200.3 ± 558	*4002.7 ± 103.7*	6506.7 ± 1541.9	5925.8 ± 610.8

Table 1: Total yield and growth of *Solanum lycopersicum* (MOMOTARO tomato) under four different cultivation conditions. Italic values and bold values for each line indicate significant differences at p<0.10 (n=3).

However, the yield and Brix yield of plants grown in UR did not increase when nutrients were supplied at EC1.0. FR cultivation with diluted nutrients (FR-EC0.5) markedly lowered shoot biomass, yield, and Brix yield. This result strongly indicated that diluted nutrients were insufficient for normal fruit production. Compared to UR-EC1.0, UR-EC0.5 decreased shoot biomass but not yield or Brix yield, suggesting that nutrients retained in UR were utilized for fruit production, thereby enabling comparable yield to FR-EC1.0. UR-EC0.5 consequently demonstrated growth and fruit yield comparable to that of FR-EC1.0. As a result, a large amount of nutrients was supplied to UR-EC1.0 and achieved the highest shoot biomass. Thus, our cultivation conditions would represent over-nutrition (UR-EC1.0) and malnutrition (FR-EC0.5).

Distinct GHG emission characteristics in different cultivation systems

It is known that nitrogen fertilization increases soil uptake of CH_4 [20], implying that over-nutrition decreased CH_4 emission when tomato was hydroponically cultivated with rockwool. Unexpectedly, seasonal CH_4 emissions (1.51 gm^{-2}) were observed only in UR-EC1.0, whereas the other cultivation plots showed absorption (Table 2).

Rockwool	CH_4 (mg m^{-2} season^{-1})		CO_2 (g m^{-2} season^{-1})		N_2O (mg m^{-2} season^{-1})	
	EC 1.0	**EC 0.5**	**EC 1.0**	**EC 0.5**	**EC 1.0**	**E C0.5**
FR	-346.8	-77.1	2067.7	1523.9	420.2	869.7
UR	1508.7	-270.3	3819.3	2341	2236.6	1039.6
Rockwool type (R)		n.s.(0.224)		*(0.016)		*(0.022)
Nutrient conc. (N)		n.s.(0.266)		*(0.044)		n.s.(0.318)
R × N		n.s.(0.143)		n.s. (0.301)		*(0.047)

Table 2: Statistical significance of the effect of Rockwool condition, nutrient concentration and the interaction of both on total GHGs emissions. Values in parentheses indicate the p value. * p<0.05, n.s. not significant in two-way ANOVA.

However, the differences among rockwool type, nutrient concentration, and their interaction were not statistically significant.

The UR system significantly increased CO_2 emissions (p=0.016), with 3.82 kg m^{-2} emitted from UR-EC1.0 compared to 2.07 kg m^{-2} emitted from FR-EC1.0, and 2.34 and 1.52 kg m^{-2} emitted from UR-EC0.5 and FR-EC0.5 respectively (Table 2). Significant differences were also observed between the different nutrient concentrations, with EC1.0 emitting 1.4 to 1.6 times more CO_2 than EC0.5 (p=0.044); however, there were no significant interactions between rockwool type and nutrient concentration. These results clearly suggest that microbial proliferation in rockwool during the previous growing season directly contributed to CO_2 emissions.

The highest total seasonal N_2O emission (2.24 gm^{-2}) was observed in UR-EC1.0 (Table 2). Compared to FR-EC1.0 and UR-EC0.5, UR-EC1.0 released 5.3 and 2.2 times more N_2O respectively; dilution of nutrient solution thus mitigated N_2O emissions. Plant growth, total yield, and Brix yield in UR-EC1.0 were comparable to those of the FR-EC1.0 treatment (Table 1). Unexpectedly, the lowest nitrogen application (FR-EC0.5) emitted almost twice as much N_2O as FR-EC1.0, although this difference was not statistically significant. As observed for plant growth and yield, FR-EC0.5 mimicked conditions of malnutrition. The nutrient concentration may have been too low to be absorbed by tomato roots, and the unabsorbed N compounds (NO_3^- and NH_4^+) may have elicited rhizospheric nitrification or denitrification [18]. As shown in Table 2, there was a significant interaction between rockwool type and nutrient concentration (p=0.047), so the factors inducing N_2O emissions were complex.

On average, N loss as N_2O amounted to 0.27 g out of 8.7 g (3.1%) in FR-EC1.0 (Table 3), comparable to levels reported by other researchers [21].

	FR		**UR**	
	EC 1.0	**EC 0.5**	**EC 1.0**	**EC 0.5**
Nitrogen-input (mg)	8729	3186	8729	4365
N-N_2O emission (mg)	267.4 ± 96.6	553.4 ± 304.3	**1423.3 ± 449.1**	661.6 ± 309.6
N_2O emission rate (%)	3.1 ± 1.1	**17.4 ± 9.6**	**16.3 ± 5.1**	**15.2 ± 7.1**

Table 3: Total nitrogen input and emission rate as N_2O. Bold values for each line indicate significant differences at p<0.10 (n=3).

The fact that FR-EC0.5 lost 17.4% of N supplied in solution strongly suggested that unutilized rhizospheric N compounds could be readily transformed and released as N_2O. Therefore, plants' ability to take up N from rhizosphere could be crucial for the control and mitigation of N_2O emissions. On the other hand, UR-EC1.0 released 1.4 g N as N_2O, though actual nitrogen retained inside UR was enigmatic. UR at the beginning of cultivation contained greater amounts of NO_3^- and NH_4^+, precursors of N_2O (Figure 1).

It is plausible that these nutrients were remnants from the previous season, and the rapid decrease in their concentrations coincided with N_2O emission during the first four weeks (Figure 1, phase I). Therefore, at present we cannot claim whether the N released as N_2O was derived from the nutrient inputs during the experiment, or from nutrients remaining from the previous season. Nevertheless, increased N_2O emission from the UR system strongly suggested that excess N fertilizer residing in rockwool was transformed to N_2O rather than providing a desirable effect of additional fertilizer for tomato plants as described above (Table 1).

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Nutrient status and interactions in the rhizosphere

Over the course of the growing season, we found no statistically significant interactions in low-molecular-weight ionic components between rockwool type and nutrient treatment (Table 4).

Predictably, macro-elements ($N\text{-}NO_3^-$, $P\text{-}PO_4^-$, and K^+) demonstrated significantly different fluctuations between nutrient concentrations.

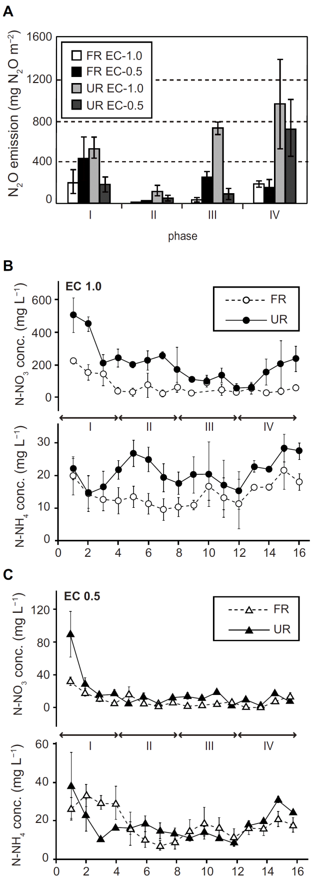

Figure 1: Comparison of the following parameters between four different cultivation treatments: (A) N_2O emissions; (B) $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ in EC1.0; and (C) $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ in EC0.5. Error bars represent the standard error of the mean. FR: freshly prepared rockwool, UR: used rockwool, EC1.0: normal levels of nutrient addition, EC0.5: low levels of nutrient addition. Data of each phase is sum of 4 wk, I: 1 to 4 wk, II: 5 to 8 wk, III: 9 to 12 wk and IV: 13-16 wk.

Rockwool type also showed significance in NO_3–, NH_4^+, PO_4^-, and K^+, confirming that UR retained soluble forms of macro-elements unabsorbed during the last growing season. However, there were neither strong nor significant Spearman's rank correlations between N_2O emission and PO_4^-, with the maximum coefficient (-0.39) at FR-EC1.0; between N_2O emission and K^+, the maximum coefficient (-0.25) was at FR-EC1.0. Despite the fact that NO_3^- and NH_4^+ are substrates for N_2O production, neither demonstrated a prominent correlation to N_2O emission (Table 5).

Even when data obtained from different cultivation periods were analyzed separately, no strong or significant correlations between N-nutrient concentrations and N_2O emissions were observed (data not shown). These results strongly suggest that remnant N compounds do not act as determinants for N_2O emission, although they undoubtedly increase the potential amount of N_2O emission.

Absorptivity of nutrients is generally dependent on mutual interactions in the rhizosphere. For example, excessive amounts of redox-active microelements (e.g., iron, zinc, and copper) can cause root oxidative stress via generation of reactive oxygen species (ROS). Then, antioxidant defense systems that can protect cells from oxidative damage and scavenge harmful ROS consume reduced forms of nicotinamide adenine dinucleotide (NADH) and thereby decrease the amount of reductant power available to reduce NO_3^- and NO_2^- to NH_4^+ in root cells. Because the concentrations of intracellular NO_3^- and NH_4^+ are directly involved in feedback processes regulating their transport systems, the status of microelements in the rhizosphere may influence N_2O emission by disturbing plants' absorption of NO_3^- and NH_4^+.

To explore underlying factors correlated with N_2O emissions in rockwool cultivation (hydroponics), fluctuations in total macro- and microelements (potassium, calcium, sodium, phosphorus, magnesium, iron, zinc, manganese, copper, boron, aluminum) and relative water content (RWC) in FR and UR were investigated. Contrary to our expectations, there were no strong correlations between these microelements and N_2O emissions (data not shown). However, we found significant and moderate correlations between %RWC and N_2O emissions in the FR system and in UR-EC0.5 (Table 5). High %RWC generally indicates anaerobic conditions in the rhizosphere, allowing rhizospheric microbial denitrification to proceed [22]. Because denitrification is a respiratory process used as an alternative to oxygen respiration under low oxygen or anoxic conditions [23], a steady state characterized by higher water content in the rhizosphere may cause anoxic conditions and trigger denitrification processes in rockwool. An exception was the UR-EC1.0 treatment, which demonstrated no correlation to %RWC, as %RWC is just one of numerous triggering factors or antecedents to the onset of N_2O emissions.

	Cl^-	NO_3^-	PO_4^-	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
R	n.s.(0.317)	**(0.002)	*** (0.000)	n.s. (0.171)	n.s.(0.677)	*** (0.000)	*** (0.000)	n.s. (0.491)	n.s. (0.549)
N	*(0.043)	**(0.003)	***0.000	n.s.(0.178)	** (0.009)	n.s.(0.205)	*** 0.000	n.s.(0.171)	n.s.(0.253)

R x N	n.s.(0.448)	n.s. (0.508)	n.s.(0.113)	n.s.(0.799)	n.s.(0.448)	n.s.(0.693)	n.s.(0.243)	n.s.(0.309)	n.s.(0.532)

Table 4: Statistical significance of the effect of Rockwool condition, nutrient concentration and the interaction of both on ion dynamics in Rockwool. R: Rockwool condition, N: nutrient concentration. Values in parentheses indicate the p value. * p<0.05, ** p<0.01, *** p<0.001, n.s. not significant in two-way ANOVA.

N_2O emission		% RWC		$N\text{-}NO_3^-$		$N\text{-}NH_4^+$	
		cc	p	cc	p	cc	p
FR	EC 1.0	0.409	0.003**	-0.095	0.506	-0.147	0.305
	EC 0.5	0.378	0.007**	-0.194	0.172	0.052	0.714
UR	EC 1.0	0.023	0.875	0.039	0.783	-0.031	0.831
	EC 0.5	0.275	0.051†	-0.012	0.936	0.113	0.428

Table 5: Spearman's rank correlation between N_2O emissions and Rockwool % RWC, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ for four different cultivations. cc: correlation coefficient, †p<0.1, *p<0.05, **p<0.01, ***p<0.001

Conclusion

Our findings have demonstrated that a considerable range of remnant nutrients, such as NO_3^- and NH_4^+, in the rhizosphere in hydroponic rockwool systems, are insufficient to promote N_2O emission, despite their importance to N_2O production. Our result provided a possibility that an anaerobic condition in rhizosphere could control N_2O production via denitrification pathway in the presence of remnant N-nutrient. So, an aerobic condition may be undesirable for mitigating N_2O emission, suggesting the importance of management of drainage in rockwool. We conclude that environmental factors dominate the fates of available environmental substrates retained in rockwool, thereby controlling N_2O fluxes. Our study does not exclude other rhizospheric environmental factors (e.g., temperature, pH, EC, dissolved oxygen, and oxidation-reduction potential), which were not assessed in this study. It is challenging but necessary to determine the most influential factors in N_2O emissions and to establish cultivation-management protocols to mitigate these emissions. Simultaneous real-time monitoring of N_2O fluxes and of environmental factors that affect these fluxes will assist in progressing toward this goal.

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