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LETTER

Irrigation with oxygen-nanobubble water can reduce methane emission and arsenic dissolution in a flooded rice paddy

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Supplementary material for this article is available online

Abstract

A remarkable feature of nanobubbles ($<10^{-6}$ m in diameter) is their long lifetime in water. Supplying oxygen-nanobubbles (NBs) to continuously flooded paddy soil may retard the development of reductive conditions, thereby reducing the emission of methane (CH_4), a potent greenhouse gas, and dissolution of arsenic, an environmental load. We tested this hypothesis by performing a pot experiment and measuring redox-related variables. The NBs were introduced into control water (with properties similar to those of river water) using a commercially available generator. Rice (*Oryza sativa* L.) growth did not differ between plants irrigated with NB water and those irrigated with control water, but NB water significantly ($p < 0.05$) reduced cumulative CH_4 emission during the rice-growing season by 21%. The amounts of iron, manganese, and arsenic that leached into the drainage water before full rice heading were also reduced by the NB water. Regardless of the water type, weekly-measured CH_4 flux was linearly correlated with the leached iron concentration during the rice-growing season ($r = 0.74$, $p < 0.001$). At the end of the experiment, the NB water significantly lowered the soil pH in the 0–5 cm layer, probably because of the raised redox potential. The population of methanogenic Archaea (*mcrA* copy number) in the 0–5 cm layer was significantly increased by the NB water, but we found no correlation between the *mcrA* copy number and the cumulative CH_4 emission ($r = -0.08$, $p = 0.85$). In pots without rice plants, soil reduction was not enhanced, regardless of the water type. The results indicate that NB water reduced CH_4 emission and arsenic dissolution through an oxidative shift of the redox conditions in the flooded soil. We propose the use of NB water as a tool for controlling redox conditions in flooded paddy soils.

1. Introduction

Paddy soil biogeochemistry is governed by the sequential reduction of soil oxidants, namely, oxygen (O_2), nitrate (NO_3^-), manganese (Mn(IV)), iron (Fe(III)), and sulfate (SO_4^{2-}), in waterlogged soil. The development of reductive soil conditions inhibits disease damage by aerobic microorganisms, which allows rice to be continuously cultivated in the same field for a very long time. Soil reduction also has negative environmental consequences. Methane (CH_4), a potent greenhouse gas, is produced in the soil and is emitted to the atmosphere (Holzapfel-Pschorn *et al* 1985). Arsenic (As), a toxic element, is solubilized and then taken up by rice in a reduced inorganic form (As^{3+}) (Takahashi *et al* 2004, Arao *et al* 2009). The use of water-management practices that supply molecular O_2 to the soil, such as midseason drainage and alternate wetting and drying, can potentially address these two problems (Arao *et al* 2009, Linquist *et al* 2015); however, implementation of these practices is not always feasible. (1) Rice has growth stage-specific water demands, (2) rice growth can be inhibited by excessive soil drying, (3) flooding is necessary to warm the soil for the rice under cool weather conditions, and (4) rainy weather may cause

Table 1. Characteristics of the tested soil and of the irrigation water.

Soil		Water	
pH (1:2.5)	6.8	pH	8.17
EC (mS m^{-1})	11	EC (mS m^{-1})	33.2
Total C (g kg^{-1})	6.3	Na^+ (mg L^{-1})	29.7
Total N (g kg^{-1})	0.3	K^+ (mg L^{-1})	6.2
Available N (mg kg^{-1})	7.6	Mg^{2+} (mg L^{-1})	4.1
CEC (cmol(+) kg^{-1})	17.8	Ca^{2+} (mg L^{-1})	18.7
Free Fe^{3+} (g kg^{-1})	10.4	Cl^- (mg L^{-1})	80.2
Texture	clay loam	Br^- (mg L^{-1})	27.3
Sand (%)	48.2	NO_3^- (mg N L^{-1})	nd
Silt (%)	28.4	HCO_3^- (mg C L^{-1})	8.0
Clay (%)	23.4	SO_4^{2-} (mg S L^{-1})	nd

CEC, cation exchange capacity; nd, not detected.

continuous flooding, especially in flood-prone rainfed lowlands in Asia. If additional O_2 could be supplied to a continuously flooded paddy soil, however, overcoming the limitations associated with existing water-management practices might be possible.

Nanobubbles ($<10^{-6}$ m in diameter) offer a means of meeting the challenge of simultaneously supplying water and O_2 to flooded paddy soil. Nanobubbles have several unique properties, including long-term stability (e.g., several to 70 days) in water (Ushikubo *et al* 2010) owing to the negatively charged surface of the bubbles (Takahashi *et al* 2007), and high gas solubility in liquids owing to the high internal pressure of the bubbles (Eriksson and Ljunggren 1999). Furthermore, free radicals generated by the collapse of nanobubbles and microbubbles (usually from 10^{-4} to 10^{-6} m in diameter) (Takahashi *et al* 2007, Matsuno *et al* 2014) have been shown to degrade organic carbon in wastewater (Li *et al* 2009a) and to inactivate microorganisms (Chu *et al* 2008, Hayakumo *et al* 2014). Recently, nanobubbles have been shown to also affect biological processes (Ebina *et al* 2013, Liu *et al* 2013).

We therefore hypothesized that irrigation with oxygen-nanobubble (hereafter referred to as NB) water, by retarding soil reduction, could reduce CH_4 emission and As dissolution in continuously flooded paddy soil. We tested this hypothesis and examined other possible effects of NBs by performing a pot experiment in which pots with and without rice plants were irrigated with NB water or control (CT) water. We measured the emissions of three greenhouse gases (CH_4, nitrous oxide (N_2O), and carbon dioxide (CO_2)), the leaching of redox products, rice growth, and microbial abundance. The results demonstrated for the first time that NB water can be used to maintain less reductive conditions in flooded paddy soil.

2. Methods

2.1. Experimental set-up

A pot experiment was carried out in the glasshouse at the National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (36.026319°N, 140.114101°E) in 2014. We used Gleyic Fluvisol (WRB classification), which is the dominant paddy soil in Japan; the soil was a little oligotrophic because it was obtained from a field that had been fallow for a long period (table 1). The mean air temperature in the glasshouse during the experimental period (20 May to 12 September) was 22.4 °C, which was 0.5 °C higher than the mean outside air temperature. No pots received any rainfall.

We installed an automated pot drainage system to reproduce vertical water percolation under field conditions. Each cylindrical pot was 30 cm high and had a surface area of 200 cm^2. Each pot was filled from the bottom up with 0.7 kg of gravel to form a 2.5 cm layer and 3.5 kg of air-dried soil to form a 17.5 cm layer; the remaining 10 cm was available for surface water. A drain hole on the bottom sidewall was connected to a peristaltic pump (EW-07553-80, Cole-Parmer, IL, USA) via a silicon tube. The drainage water was collected in a tank, which was exposed to air. Drainage water that had not been exposed to air was sampled via a port halfway along the drain tube and used for analyses of dissolved greenhouse gases and leached redox metals.

2.2. Preparation of irrigation water

We prepared the irrigation water by mixing deionized water with chemicals to reproduce the major water characteristics of nearby rivers (Yabusaki *et al* 2006), including pH, electrical conductivity (EC), and Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and HCO_3^- concentrations. However, we did not add NO_3^- and SO_4^{2-}, because they are major oxidants in paddy soils. To make the CT water, we mixed 30 L of deionized water with 1.88 g $CaCl_2$, 2.35 g Na_2CO_3, 0.235 g K_2CO_3, 0.94 g $MgBr_2$, and 2.0 mL HCl (35–37% concentration) (table 1).

To make the NB water, we used a nanobubble generator (DBON, Tashizen Techno Works, Kumamoto, Japan) to introduce O_2 (purity 99.99995%) into an aliquot of CT water for 1 h while keeping the water temperature at 20–25 °C. The generator was operated for 1 h before each irrigation of the pots receiving NB water. The subsequent observations of the NB water indicated that it contained NBs. We did not observe the milky-white color that is specific to microbubble water (Takahashi *et al* 2007) during or after the preparation of the NB water. Electron spin resonance (ESR) spectra measured using an ESR spectrometer (ESRX-10SA-v4, Keycom, Tokyo, Japan) following the method of Takahashi *et al* (2012) clearly showed the presence of free radicals likely generated by the collapse of NBs (figures 1(a) and (b)). The characteristics of the NB water did not differ from those of the CT water (table 1), except that the concentration of dissolved O_2 (DO) was higher for a few days after NB generation under gentle stirring conditions (figure 1(c)).

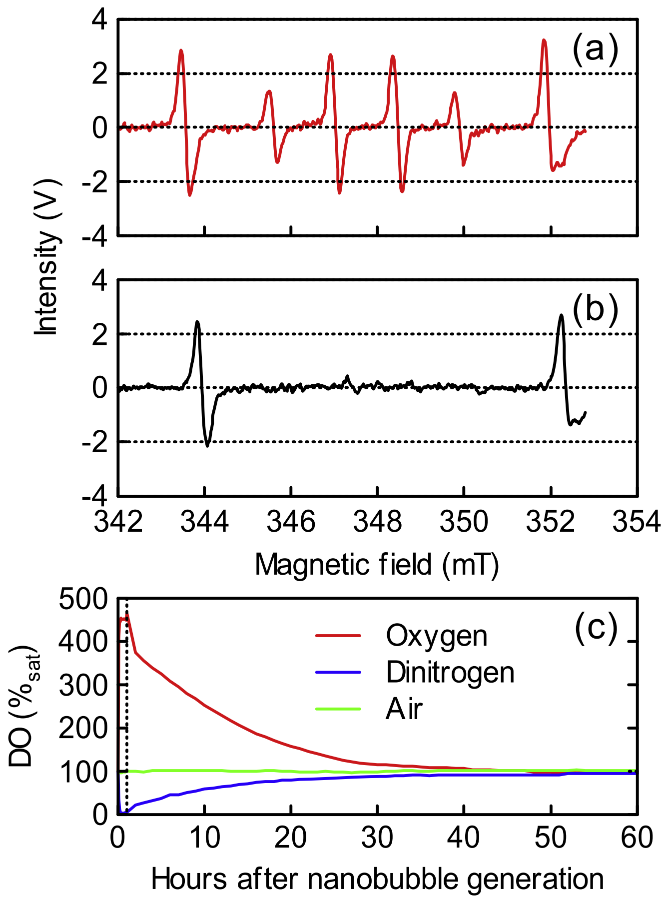

Figure 1. Properties of NB water. ESR spectra of (a) NB water and (b) CT water. (c) Temporal shift in DO concentration during and after the generation of NBs. The vertical dotted line indicates the end of the 1 h generation period. DO data for nanobubbles of dinitrogen and air are shown for reference. The DO concentration is expressed as the percent saturation at the water temperature when measured ($\%_{sat}$). See main text for the detailed methods.

The highest observed DO concentration (40.0 mg L^{-1} at 20.1 °C) was comparable to the theoretical maximum (42.1 mg L^{-1} at 20 °C). The water's pH was temporarily increased by 0.04 units from the initial value of 8.36 during the preparation of the NB water, and then converged to 8.17. The initial increase was likely as a result of a decrease in the partial pressure of CO_2, an effect also observed by Liu *et al* (2013).

2.3. Rice cultivation

The pot experiment consisted of two factors: water type (CT or NB) and rice planting (*Oryza sativa* L. cv. Koshihikari) (with (+R) or without (–R)). We performed four replications for each of the four treatments (i.e., a total of 16 pots). One day before rice seedlings were transplanted, air-dried soil was mixed with chemical fertilizer and 5 g of rice straw cut into pieces 1–2 cm long, and then puddled with deionized water. The basal fertilizer consisted of urea, fused magnesium phosphate, and potassium chloride, and the application rates were 0.3 g N pot^{-1}, 0.3 g P_2O_5 pot^{-1}, and 0.3 g K_2O pot^{-1}. Three rice seedlings (21 days old) were transplanted into each +R pot on 20 May. Irrigation and automated drainage were started 1 day after transplanting (DAT), and all pots were then kept continuously flooded until 105 DAT. The drainage rate was set at 10 mm d^{-1} (200 mL pot^{-1} d^{-1}), which is the normal rate in Japanese paddy fields. Topdressing of urea was applied twice, at 29 and 49 DAT, each time at a rate of 0.3 g N pot^{-1}. Weeds were carefully removed when they were small. The aboveground parts of the rice plants were harvested when the plants reached maturity (12 September, 115 DAT).

2.4. Measurements

We measured fluxes of CH_4 and N_2O from the soil to the atmosphere by using a transparent static chamber (30 cm long × 30 cm wide × 60 cm high with an extension column of the same size). Under +R conditions, the measurements were performed weekly, or more often after N topdressing and final drainage, from 09:30 to 10:30 (local time) (Minamikawa *et al* 2012). Under –R conditions, the measurements were performed monthly. A gas sample was collected 1, 11, and 21 min after closure of the chamber with a water seal. The concentrations of CH_4 and N_2O in the samples were analyzed using two different gas chromatographs. Dissolved CO_2, CH_4, and N_2O concentrations in the drainage water were measured weekly by a headspace gas sampling technique (Minamikawa et al 2010). The cumulative amount of gas dissolution was calculated by integrating the amount between two consecutive measurements (i.e., dissolved gas concentration × amount of drainage water).

The drainage water samples for the analyses of heavy metals were passed through a 0.20 μm membrane filter and acidified just after collection. The concentrations of Fe and Mn were analyzed biweekly by using inductively coupled plasma–optical emission spectrometry (720-ES, Agilent, CA, USA). The concentration of As was determined biweekly by flow injection–inductively coupled plasma mass spectroscopy (NexION 300XX; Perkin-Elmer Sciex, MA, USA) with a minor modification as described by Baba et al (2014).

The pH, EC, and amount of stored tank water were recorded weekly. An aliquot of the water was passed through a 0.20 μm membrane filter and subjected to instrumental analyses. The concentrations of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were determined by a combustion-infrared method (TOC-V, Shimadzu, Kyoto, Japan). The concentrations of cations (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}) and anions (Cl^-, Br^-, $NO_3{}^-$, and $SO_4{}^{2-}$) were determined by ion chromatography (ICS-1600, Thermo Scientific Dionex, CA, USA). The CO2SYS program was used to compute the concentration of HCO_3^- from the measured pH and DIC values (Pierrot et al 2006). We did not record the amount of irrigation water applied, but used the leached Br^- amount as a proxy for it because the tested soil did not initially contain Br^-.

The redox potential (ORP_{SHE}) at a soil depth of 5 cm was measured weekly by using three preinstalled platinum-tipped electrodes and a portable meter with a silver–silver chloride reference electrode (PRN-41, Fujiwara Scientific, Tokyo, Japan). The contents of total carbon and nitrogen at soil depths of 0–5 and 5–10 cm after harvest (115 DAT) were determined by the dry combustion method (NC-22, Sumika Chemical Analysis Service, Tokyo, Japan). Soil pH (soil: water, 1:2.5) at 0–5 cm depth was measured just before transplanting (0 DAT) and just before final drainage (104 DAT).

Using the same soil samples as those for pH measurement, we quantified the methanogenic archaeal *mcrA* gene (α subunit of methyl-coenzyme M reductase) and the methanotrophic bacterial *pmoA* gene (α subunit of the particulate methane monooxygenase) by real-time quantitative polymerase chain reaction analysis following the methods of Watanabe et al (2010) and Kolb et al (2003), respectively. We confirmed that the initial soil pH (6.88 ± 0.03 (1σ)), *mcrA* copy number ($3.11 \pm 1.57 \times 10^6$ copies g^{-1} dry soil), and *pmoA* copy number ($3.49 \pm 2.13 \times 10^4$ copies g^{-1} dry soil) at 0 DAT were not biased among pots. In addition, we prepared four extra pots (one per treatment) in which we measured the seasonal hourly soil temperature at 5 cm depth and the soil pH at 51 DAT (a seasonal midpoint).

We recorded the plant height and the number of tillers/ears in the +R pots biweekly, and we also recorded the heading date (i.e., 50% ear emergence), which was determined by frequent observation. After harvest, we measured the dry weight of the harvested aboveground parts.

2.5. Statistical analysis

We used two-way analysis of variance (ANOVA) to analyze the effects of water type (CT or NB) and rice planting (+R or –R) on the measured variables. We computed Pearson's correlation between the measured variables to explore the mechanisms underlying the reduction of CH_4 emission by the use of NB water. All computations were performed using JMP 8.0 software (SAS Institute, NC, USA). The significance level was set at $p < 0.05$ for all statistical tests.

3. Results and discussion

3.1. Rice growth and the surrounding environment

NB water had no observable effect on rice growth. This result is inconsistent with the findings of previous studies reporting a positive effect on biological processes (Ebina et al 2013, Liu et al 2013). Plant height and tiller/ear number were normal regardless of water type (figure 2). The heading date was 78 ± 3 (min/max) DAT. The harvested aboveground biomass did not differ significantly between NB and CT water (63.0 ± 1.9 (1σ) g dry-weight pot^{-1}, $n = 8$), although the grain filling was slightly poor in all +R pots. None of the plant parameters (height, maximum tiller number, ear number, or biomass) significantly correlated with either cumulative direct CH_4 emission to the atmosphere or the cumulative amount of CH_4 dissolution in the drainage water ($p = 0.11$–0.98).

Surrounding environmental data support the finding of uniform rice growth between NB and CT water. The pump drainage system worked well, although clogging occurred occasionally. The cumulative amount of drainage water was thus slightly lower (on average 3.5%) than planned, but it did not differ among the four treatments (table 2). The cumulative amount of irrigation water was estimated to be 2078 mm under +R conditions and 1327 mm under –R conditions. The 57% greater amount under +R conditions is mainly attributable to rice transpiration. Accordingly, the supply of NBs to the soil was also likely to be greater under +R conditions. Soil temperature at 5 cm depth was almost the same among the four extra pots, and the seasonal mean (23.7 °C) was 1.3 °C higher than the seasonal mean air temperature.

3.2. Seasonal CH_4 dynamics

As hypothesized, the use of NB water under +R conditions significantly reduced, by 21%, the

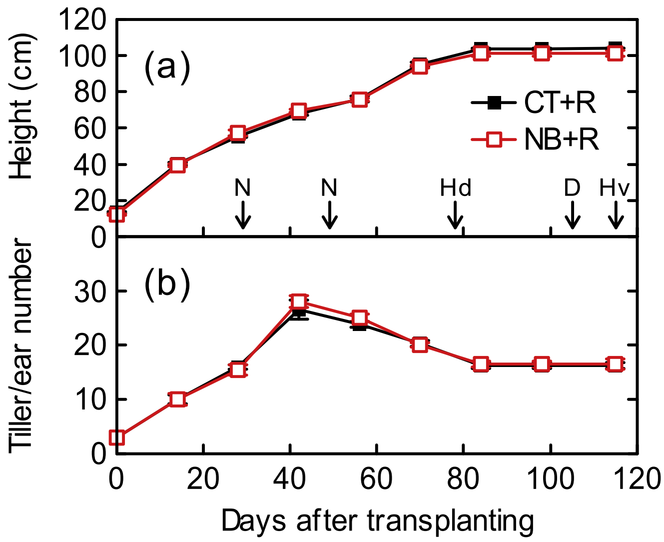

Figure 2. Seasonal variations in (a) rice height and (b) tiller/ear number. Bars indicate standard errors ($n = 4$). N, urea topdressing; Hd, heading; D, final drainage; Hv, harvest.

Table 2. Cumulative amounts of drainage water, leached ions and metals, and CH_4 emission and dissolution as affected by water type (W) and rice planting (P).

Treatment	Drainage (mm)	Br^- (mg pot^{-1})	NO_3^- (mg N pot^{-1})	SO_4^{2-} (mg S pot^{-1})	Mn (mg pot^{-1})	Fe (mg pot^{-1})	As (μg pot^{-1})	CH_4 emission (mg CH_4 pot^{-1})	CH_4 dissolution (mg CH_4 pot^{-1})
CT+R	1015	1133	4.07	326	133.0	142.4	247.6	545.0 a	32.7
NB+R	1046	1136	4.04	332	123.1	140.7	230.5	430.2 b	32.2
CT–R	995	719	4.43	350	28.3	2.4	36.4	12.3 c	45.4
NB–R	1043	731	4.39	347	31.7	2.6	37.0	3.2 c	53.1
ANOVA									
W	ns	ns	ns	ns	ns	ns	ns	*	ns
P	ns	***	ns	*	***	***	***	***	**
W × P	ns	ns	ns	ns	ns	ns	ns	*	ns

Different letters indicate a significant difference by Tukey's honestly significant difference test ($p < 0.05$) when the W × P interaction was significant.

***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

cumulative direct CH_4 emission to the atmosphere (table 2). The seasonal CH_4 flux pattern was similar in the NB+R and CT+R treatments (figure 3(a)); the gradual increase in flux observed during the rice-growing season is typical for rice paddies in Japan with low organic amendment (Yagi et al 1996, Tokida et al 2010). By contrast, under –R conditions, the CH_4 flux remained near zero throughout the flooded period (i.e., before 105 DAT; figure 3(a)). This is because –R conditions lacked both a plant-derived carbon substrate for CH_4 production (root exudates and debris) and the dominant pathway of direct CH_4 emission (rice aerenchyma). These different responses to water type between +R and –R conditions explain the significant interaction between the water type and rice planting (table 2).

Contrary to expectation, the cumulative amount of CH_4 dissolution in the drainage water did not differ between the NB and CT water under +R conditions (table 2). No difference was detected mainly because the dissolved CH_4 concentration in the CT+R treatment fell sharply after 88 DAT (shaded area in figure 3(b)). From 49 to 88 DAT, however, the dissolved CH_4 concentration was significantly lower in the NB+R treatment than in the CT+R treatment. We discuss the reason for the sharp fall after 88 DAT in section 3.3. Here, we note, however, that this fall was not reflected in the CH_4 flux (figures 3(a) and (b)). A possible explanation for this inconsistency after 88 DAT (table 3) is the decrease in CH_4 transport capacity (conductance) of rice due to senescence (Nouchi et al 1994). Cheng et al (2008) observed a positive relationship between the CH_4 flux and the dissolved CH_4 concentration in soil solutions before heading, which is consistent with our results, but the CH_4 flux decreased with increasing CH_4 concentration after heading. Another explanation is an increase over time in the fraction of gas-phase CH_4 in the total CH_4 pool of flooded paddy soil (Tokida et al 2013). The volume of bubbles can affect the rate of plant-mediated CH_4

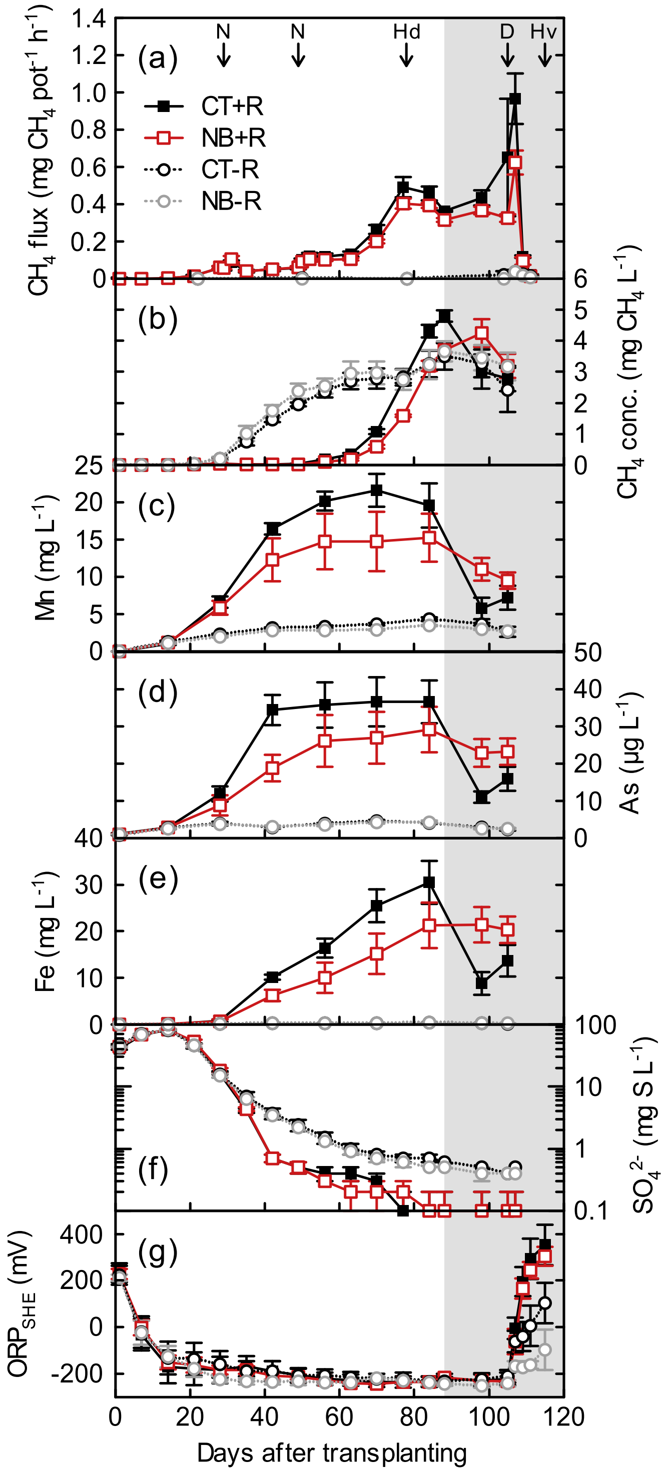

Figure 3. Seasonal variations in (a) CH_4 flux, the concentrations of (b) dissolved CH_4, (c) Mn, (d) As, (e) Fe, and (f) SO_4^{2-} in the drainage water, and (g) ORP_{SHE} at a soil depth of 5 cm. Bars indicate standard errors ($n = 4$). N, urea topdressing; Hd, heading; D, final drainage; Hv, harvest. Shading indicates the period after 88 DAT.

emission by enhancing the diffusive uptake of CH_4 by the rice roots (Tokida *et al* 2013). The ratio of the cumulative amount of CH_4 dissolution in the drainage water to the cumulative direct CH_4 emission to the atmosphere under +R conditions is comparable to the ratio obtained previously in a pot experiment (Murase and Kimura 1996). Under –R conditions, the seasonal pattern and magnitude of the dissolved CH_4 concentration were almost the same between the NB and CT water (figure 3(b)). This was likely because the supply of NBs under –R conditions was not sufficient, as estimated in section 3.1, to decrease the amount of CH_4 dissolved in the drainage water. The earlier rise in dissolved CH_4 concentration under –R conditions than under +R conditions can be explained by the lack of rice aerenchyma, as described above.

Table 3. Pearson's correlation coefficients between CH_4 flux and redox-related variables before 88 DAT and from 88 to 115 DAT under +R conditions.

Variable	Before 88 DAT ($n = 56$)	88–115 DAT ($n = 16$)	All days ($n = 72$)
Dissolved CH_4 concentration	0.93^{***}	-0.11^{ns}	0.90^{***}
Leached Mn concentration	0.66^{***}	-0.25^{ns}	0.43^{***}
Leached As concentration	0.66^{***}	-0.28^{ns}	0.47^{***}
Leached Fe concentration	0.86^{***}	-0.35^{ns}	0.74^{***}
ORP_{SHE} at 5 cm depth	-0.46^{***}	0.08^{ns}	-0.49^{***}

***, $p < 0.001$; ns, not significant.

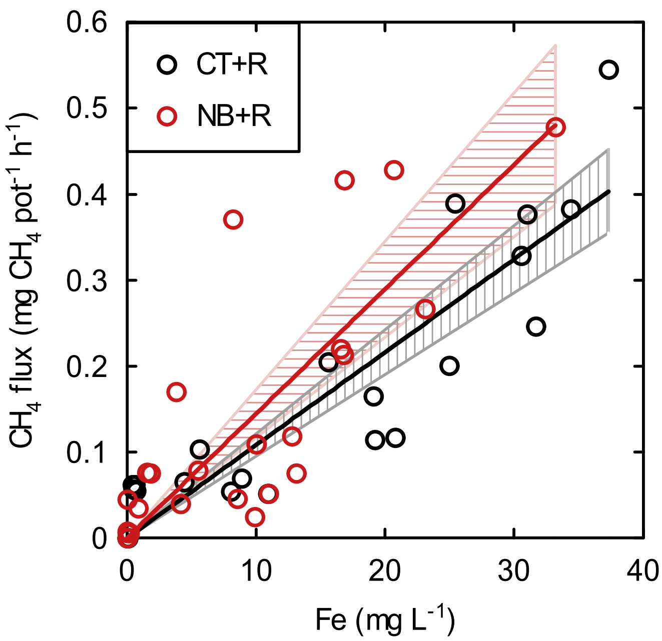

Figure 4. Linear regression between Fe concentration in the drainage water and CH_4 flux in the NB+R and CT+R treatments before 88 DAT. Shaded areas indicate the 95% confidence intervals of the slopes. Both intercepts were statistically insignificant and thus regarded as zero.

3.3. Oxidative shift in redox conditions due to NB water

The amounts of leached redox metals support our hypothesis that NB water can result in less reductive conditions in flooded paddy soils. Although the effect of water type on the cumulative amount of leached Mn, As, or Fe was not significant (table 2), the concentration of each metal at four consecutive measurement times from 42 to 84 DAT was significantly lower in the NB+R treatment than in the CT+R treatment (figures 3(c), (d), and (e)). Arsenic availability of rice plants increases with increasing As concentration in the soil solution (Marin *et al* 1993, Li *et al* 2009b). Therefore, although the present study did not measure the As content in the harvested rice aboveground, the irrigation with NB water may reduce the As uptake by rice plants. The concentration of leached $SO_4{}^{2-}$, which was not applied experimentally, declined earlier under +R conditions, but was not affected by water type (figure 3(f) and table 2). The concentration of leached $NO_3{}^-$, which was also not applied, declined to an undetectable level by 14 DAT and did not differ among treatments (table 2). Tokida *et al* (2010) reported a tight stoichiometric competition for electron donors between Fe(III) reduction and CH_4 production in flooded paddy soil. In the present study, each of the leached Fe, Mn, and As concentrations in the drainage water was significantly correlated with the CH_4 flux under +R conditions, especially up to 88 DAT (table 3). Furthermore, the slope of the linear regression of the CH_4 flux against the leached Fe concentration up to 88 DAT did not differ for the two water types (figure 4), suggesting that the stoichiometric relationship was the same in both the NB+R and CT+R treatments. The seasonal pattern of ORP_{SHE} at a soil depth of 5 cm was, however, similar among treatments (figure 3(g)). Even if ORP_{SHE} is low enough for soil As to be reduced, microbial activity is necessary for the reduction to take place (Yamaguchi *et al* 2011), and the same is true for the reduction of

Table 4. Soil pH, *mcrA* and *pmoA* copy numbers, and the total carbon and nitrogen contents at a soil depth of 0–5 cm at 104 DAT as affected by water type (W) and rice planting (P).

Treatment	pH	*mcrA* (10^7 copies g^{-1} dry soil)	*pmoA* (10^5 copies g^{-1} dry soil)	Total C ($g\ kg^{-1}$ dry soil)	Total N ($g\ kg^{-1}$ dry soil)
CT+R	7.01	2.92	11.84	6.59	0.31
NB+R	6.51	4.08	7.11	6.75	0.35
CT–R	7.07	0.54	2.20	6.60	0.41
NB–R	6.58	1.07	1.14	6.63	0.39
ANOVA					
W	***	*	ns	ns	ns
P	ns	***	*	ns	***
W × P	ns	ns	ns	ns	ns

***, $p < 0.001$; *, $p < 0.05$; ns, not significant.

soil Mn and Fe. The negligible amounts of leached redox metals under –R conditions (figures 3(c), (d), and (e) and table 2) can thus be explained by the limited supply of carbon substrate for microbial activity in the –R pots.

The lack of a difference in the ORP_{SHE} at a 5 cm depth raises a fundamental question: to what depth in the flooded soil did the NBs have an effect? In the present study, the soil pH data provide indirect evidence for the depth of the effect. The pH of the 0–5 cm soil layer at 104 DAT was significantly lowered for the NB water (table 4). This result is consistent with the pH observed in the four extra pots at 51 DAT (i.e., 6.52–6.60 for NB water versus 7.05–7.12 for CT water). Dorau and Mansfeldt (2015) reported the increase in pH of a soil suspension simultaneous with the artificial decrease in ORP_{SHE} by the addition of dinitrogen gas in a microcosm experiment. We therefore speculate that the acidic shift of the soil pH associated with the use of NB water resulted from an increase in soil ORP_{SHE} within the 0–5 cm depth range. The ORP_{SHE} in the $Fe^{2+}/Fe(OH)_3$ system can be calculated by using the Nernst equation as follows: ORP_{SHE} (V) = 0.931−0.059 log $[Fe^{2+}]$ (activity)−0.177 × pH. This would be the dominant redox reaction under the experimental conditions because Fe (III) (hydr)oxides are the predominant oxidants of paddy soils. According to this equation, and if it is assumed that the soil pH is governed only by the soil ORP_{SHE}, then the decrease in pH of 0.5 units should theoretically increase ORP_{SHE} by ~89 mV. Further investigations that include high-resolution soil profiling will be necessary to elucidate the penetration depth of NB water, as well as other possible causes of the observed change in soil pH.

Why did the dissolved CH_4 concentration in the CT+R treatment fall sharply after 88 DAT (see figure 3(b))? As a prerequisite, rice senescence would have commonly affected the soil biogeochemistry in both the NB+R and CT+R treatments. Sharp decreases similar to that of the dissolved CH_4 concentration at this time were observed, not only in the concentrations of Mn, As, and Fe (figures 3(c), (d), and (e)) but also in the dissolved CO_2 concentration (figure 5(e)). Generally, the concentrations of Fe(II) and Mn(II) in flooded paddy soil, after an initial increase, reach a plateau, after which they do not change (Inubushi *et al* 1984, Tokida *et al* 2010). However, the concentrations of Fe^{2+} and Mn^{2+} in soil leachate can in fact decline after peaking (e.g., Kimura *et al* 1992), in part as a result of the precipitation of compounds such as metal sulfides and carbonates (Gao *et al* 2002). A plausible explanation for the sharp decline of the above-mentioned variables in the CT+R treatment is the limited availability of plant-derived carbon substrate to act as electron donor for the sequential biological reduction of soil oxidants. Because a weakly acidic (5.5–6.5) soil pH is optimal for rice growth, the slightly higher pH in the CT+R treatment (table 4) may have decreased the rice metabolic activity. If so, irrigation water characteristics and soil types other than those used in the present study may yield different results for rice growth.

3.4. Were there any other effects of NB water?

Although previous studies have reported an effect of nanobubbles on microbes and organic compounds (Chu *et al* 2008, Hayakumo *et al* 2014, Li *et al* 2009a), we did not find any evidence for enhanced degradation of soil organic carbon associated with the use of NB water. The total carbon and nitrogen contents at 115 DAT did not differ significantly between the NB and CT water at soil depths of 0–5 cm (table 4) or 5–10 cm (data not shown). Similarly, the water type did not affect the cumulative amount of CO_2 dissolution in the drainage water, carbon-related leachates (DOC and HCO_3^-), or water pH (figures 5(a), (c), (d), and (e) and table 5). As shown by the seasonal patterns of water EC (figure 5(b)), the leached amounts of cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and Cl^- were not significantly affected by the water type (table S1 and figure S1). Neither the cumulative direct N_2O emission to the atmosphere nor the cumulative amount of N_2O dissolution in the drainage water differed among treatments (table 5). The global warming potentials (Myhre *et al* 2013) of the cumulative direct N_2O emissions under +R conditions, in terms of CO_2 equivalents, were negligible compared to the

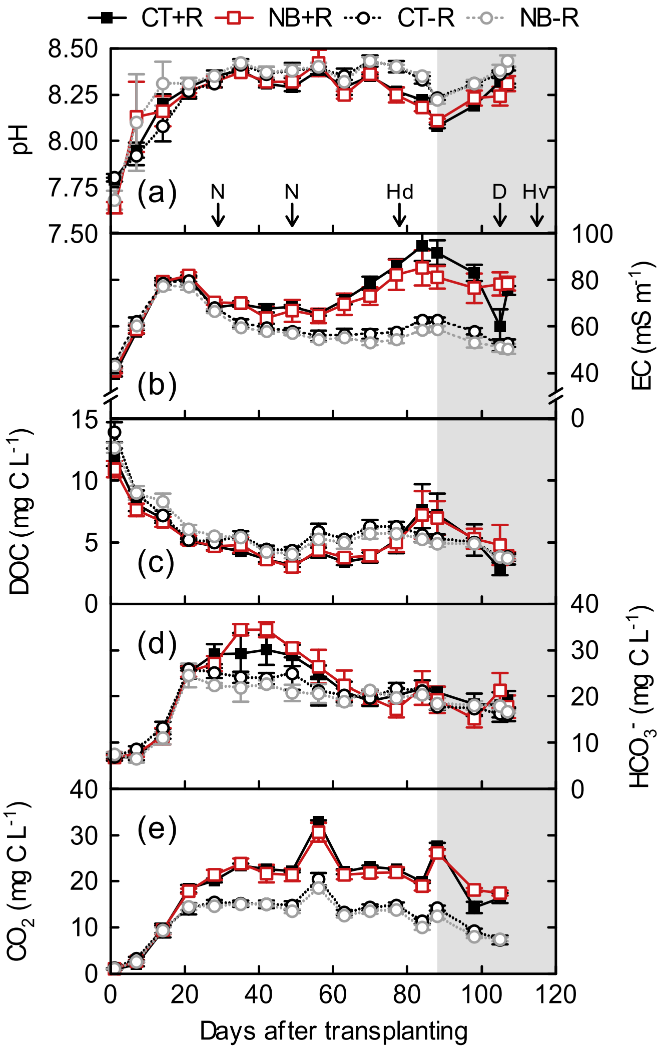

Figure 5. Seasonal variations in (a) pH, (b) EC, and the concentrations of (c) DOC, (d) HCO_3^-, and (e) dissolved CO_2 in the drainage water. Bars indicate standard errors ($n = 4$). N, urea topdressing; Hd, heading; D, final drainage; Hv, harvest. Shading indicates the period after 88 DAT.

Table 5. Cumulative amounts of N_2O emission and dissolution, CO_2 dissolution, and carbon-related leachates as affected by water type (W) and rice planting (P).

Treatment	N_2O emission (μg N pot^{-1})	N_2O dissolution (μg N pot^{-1})	CO_2 dissolution (mg C pot^{-1})	DOC (mg C pot^{-1})	HCO_3^- (mg C pot^{-1})
CT+R	520	77.9	385	108	854
NB+R	–71	85.7	403	112	895
CT–R	173	96.0	245	118	774
NB–R	314	100.2	243	121	769
ANOVA					
W	ns	ns	ns	ns	ns
P	ns	ns	***	ns	*
W × P	ns	ns	ns	ns	ns

***, $p < 0.001$; *, $p < 0.05$; ns, not significant.

corresponding CH_4 values because of the continuously flooded conditions.

Surprisingly, the NB water significantly increased the copy number of *mcrA* (i.e., the population of methanogenic Archaea) at a soil depth of 0–5 cm at 104 DAT (table 4). However, the cumulative direct CH_4 emission was not correlated with the *mcrA* copy number under +R conditions ($n = 8$, $r = -0.08$, $p = 0.85$), suggesting that the change in the methanogenic archaeal population did not cause the decline in CH_4 emission associated with NB water. Watanabe *et al* (2009) have reported that a small change in CH_4 production is not reflected in a change of methanogen population estimated by *mcrA* abundance. Thus, the reason for the increased *mcrA* copy number associated with the NB water remains unknown. In contrast, only rice planting significantly affected the *pmoA* copy number at a soil depth of 0–5 cm at 104 DAT (table 4). Reim *et al* (2012) showed by a microcosm experiment that *pmoA* gene diversity in vertical soil profiles varied with soil depth variation on the order of millimeters. Accordingly, the lack of an effect of water type on the *pmoA* copy number might be partly attributable to the use of composite samples of the 0–5 cm soil layer. High-resolution soil profiling should clarify the effect of water type on the abundance of microbes involved in CH_4 dynamics.

4. Conclusion

The present study demonstrated for the first time that irrigation with NB water can mitigate both CH_4 emission and As dissolution in flooded paddy soil. As indicated by the decreased leaching of Fe and Mn, the major soil oxidants, in the drainage water, irrigation with NB water caused redox conditions in the shallow soil layer to be more oxidative compared to irrigation with CT water. Up to now, it has been impractical to control the redox conditions in flooded paddy soil, but irrigation with NB water may offer a solution. However, the extent to which NBs penetrates flooded paddy soil was not determined in the present study. Further investigation, including high-resolution soil profiling, will be necessary to stoichiometrically explain the oxidative shift in the redox conditions associated with irrigation with NB water. For practically applying the irrigation with NB water in combination with or without other water management practices at field to landscape scale, the operating cost of commercially available large-scale NB generators and the total greenhouse gas emissions from the whole process of rice production should be evaluated in future studies.

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