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Isotopomer analysis of production, consumption and soil-to-atmosphere emission processes of N_2O at the beginning of paddy field irrigation

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ABSTRACT

In irrigated rice paddies, episodic release of nitrous oxide (N_2O) from the soil to the atmosphere has been observed during the first flood irrigation, but the biogeochemical mechanisms underlying these emissions remain unclear. To elucidate both microbial pathways of N_2O production, consumption and emission processes of N_2O from soil surfaces, we analyzed isotopomer ratios (bulk nitrogen and oxygen isotope ratios, $\delta^{15}N^{bulk}$ and $\delta^{18}O$, and intramolecular ^{15}N site preference, SP) of surface-emitted N_2O and N_2O in soil gas from paddy fields in Japan at the beginning of irrigation. Results indicate that surface-emitted N_2O is produced at shallow depths above the rising groundwater table, and that it is emitted by diffusive transport through air-filled soil pores. Immediately after soil surfaces are submerged, N_2O accumulates in the soil because of the low diffusivity and high solubility of N_2O in water. Isotopomer analysis revealed that high N_2O emissions during the flooding process resulted mainly from N_2O production by bacterial denitrification (nitrate reduction). Moreover, as soil submergence progressed, declining soil NO_3^- concentration promoted the use of N_2O as an electron acceptor. Thereby, most of the N_2O was reduced to N_2. N_2O emissions were increased by nitrogen fertilizer application before irrigation. The applied nitrogen fertilizer might enhance N_2O production.

1. Introduction

Agricultural soil is a major source of nitrous oxide (N_2O), which contributes to global warming and ozone depletion. In fact, N_2O has 298-times-higher global warming potential than carbon dioxide (CO_2) over a time horizon of 100 years (Forster et al., 2007). Microbial processes produce N_2O in soil by nitrification as a byproduct of ammonia (NH_4^+) oxidation, via hydroxylamine (NH_2OH) to nitrite (NO_2^-) in aerobic conditions, and by denitrification as an intermediate product of nitrate (NO_3^-) and NO_2^- reduction under anaerobic conditions. Subsequently, N_2O is further reduced to dinitrogen gas (N_2) by denitrification. In addition, it is known that some nitrifying bacteria produce N_2O by NO_2^- reduction similar to denitrifying bacteria, which is called as nitrifier-denitrification (Wrage et al., 2001). Although these microbial processes in soil have previously been considered mainly as attributable to bacterial processes (Zumft, 1997), fungal denitrification (Shoun and Tanimoto, 1991; Shoun et al., 1992) and archaeal nitrification (Treusch et al., 2005; Leininger et al., 2006; Nicol and Schleper, 2006; He et al., 2007) have also been reported.

High uncertainty prevails in the estimation of global N_2O emissions. Stehfest and Bouwman (2006) estimated the global annual N_2O–N emission from fertilized fields as 3.3 Tg y^{-1} with the 95% confidence interval of −51% to 107%. One reason for this variation is the episodic peak (burst) of N_2O emissions. A significant increase in N_2O emissions is found most typically after fertilizer application (e.g., Dobbie et al., 1999; Akiyama and Tsuruta, 2002, 2003; Jones et al., 2007; Hayakawa et al., 2009). However,

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episodic peaks of N_2O emissions have also been detected after rainfall (e.g., Lessard et al., 1996; Dobbie et al., 1999; Sehy et al., 2003; Nishimura et al., 2005; Kusa et al., 2006; Ellert and Janzen, 2008). Other short-lived N_2O emissions due to freezing and thawing of agricultural soils (Christensen and Tiedje, 1990; Jacinthe et al., 2002; Sharma et al., 2006) or wetting soil after drying (Rudaz et al., 1991; Cates and Keeney, 1987; Ruser et al., 2006) were also reported. Those studies reported that N_2O emissions during these events are due to an increased availability of substrates by release of available carbon by the disruption of soil aggregates and enhanced turnover of microbial biomass (Edwards and Cresser, 1992; Lundquist et al., 1999; Kalbitz et al., 2000; Sharma et al., 2006). Although those short-lived N_2O peaks account for a large portion of annual N_2O emissions, monitoring in low temporal and spatial resolution has difficulty in capturing such spiky peaks.

Elucidating the environmental variables affecting the short-lived N_2O emissions is useful for enhancing the accuracy of both N_2O monitoring and relevant numerical models. Many soil incubation studies of the effect of soil water content on N_2O emission have been conducted under several different soil water conditions (e.g., Firestone and Tiedje, 1979; Nugroho and Kuwatsuka, 1992; Weier et al., 1993; Bandibas et al., 1994; van Groenigen et al., 2005a). However, soil incubation studies have difficulty reproducing the temporal dynamic change of soil water content of actual fields. Therefore, it is also important to elucidate the influences of the rapid increase of soil water contents on temporal changes of N_2O emission or production and consumption processes of N_2O under actual field conditions.

Recent studies suggested that rice cultivation is an important anthropogenic source of atmospheric N_2O and methane (Cai et al., 1997; Akiyama et al., 2005). As for paddy rice cropping in Japan, fields are entirely submerged before rice seedling transplantation. During the flooding process, episodic release of N_2O from the soil to the atmosphere has been observed, which has been regarded as resulting from denitrification attributable to developing anaerobic conditions with subsequent rapid reduction of NO_3^- in the topsoil (Nishimura et al., 2004, 2011; Toyoda et al., 2011). However, production and consumption processes of N_2O in the soil and its emission process from soil surfaces remain unclear.

For estimation of the relative contribution of nitrification and denitrification for N_2O production or the degree of N_2O reduction to N_2, changes in the natural abundances of N and O isotopes have been used to characterize N_2O in soil ecosystems (Kim and Craig, 1993; Perez et al., 2000; Van Groenigen et al., 2005b; Wrage et al., 2005; Rock et al., 2007; Kool et al., 2010, 2011b; Minamikawa et al., 2011). In addition, variations of N and O isotopes in inorganic N species can provide important information about nitrogen cycles in ecosystems (Ostrom et al., 2002). In general, biological processes are thought to fractionate in favor of lighter isotopes relative to heavier isotopes. Fractionation during N_2O production by nitrification is generally higher than that of denitrification, which means that the N_2O produced by nitrification is more depleted in ^{15}N and ^{18}O relative to substrates than the N_2O produced by denitrification (Baggs, 2008; Park et al., 2011; Toyoda et al., 2011). However, the utility of $\delta^{15}N$ values for distinguishing nitrification and denitrification presents several complications (Baggs, 2008). First, the enrichment factors for ^{15}N for the processes depend on the compounds of substrates and on their $\delta^{15}N$ value (Mariotti et al., 1981; Barford et al., 1999; Wunderlich et al., 2012). Second, the isotopic compositions of the N_2O are influenced by the degree of N_2O reduction to N_2 (Menyailo and Hungate, 2006; Ostrom et al., 2007; Jinuntuya-Nortman et al., 2008). Third, N_2O derived from archaeal NH_4^+ oxidation has $\delta^{15}N$ values that are higher than those observed for NH_4^+ oxidizing bacteria (Santoro et al., 2011) (NH_4^+ oxidation is regarded as nitrification in this study). Similar complications arise when using $\delta^{18}O$ signatures to distinguish microbial processes of N_2O production and consumption. Moreover, measured ^{18}O enrichment factors are highly variable (Baggs, 2008; Park et al., 2011; Toyoda et al., 2011) and the $\delta^{18}O$ values of N_2O are influenced by oxygen exchange between N_2O precursors and water (Kool et al., 2007, 2009b; Casciotti et al., 2010).

In addition to these "bulk" (average) isotope ratios, analyses of intramolecular distribution of ^{15}N in N_2O (Brenninkmeijer and Rockmann, 1999; Toyoda and Yoshida, 1999) have been proposed as new tools for discerning the microbial pathways of N_2O production. In contrast to $\delta^{15}N^{bulk}$ and $\delta^{18}O$, the difference between central and terminal ^{15}N enrichment ($\delta^{15}N^{\alpha} - \delta^{15}N^{\beta} = {}^{15}N$ site preference (SP)) is considered to be independent of the isotopic signature of the substrate (Toyoda et al., 2002). Previous pure culture studies indicated that SP during N_2O production can distinguish bacterial denitrification and nitrifier-denitrification from other processes including bacterial or archaeal nitrification and fungal denitrification (Decock and Six, 2013). The reported SP values for bacterial denitrification and nitrifier-denitrification are $-2.2 \pm 3.2‰$ and $-1.0 \pm 4.3‰$, respectively, while reported SP values for N_2O produced by bacterial NH_2OH oxidation (nitrification) are $33 \pm 1.6‰$, irrespective of microbial species (Decock and Six, 2013). The SP values for produced N_2O are $30.8 \pm 4.4‰$ for archaeal nitrification (Santoro et al., 2011) with archaeal enrichment cultures (Santoro et al., 2010), and $37.0 \pm 2.3‰$ for fungal denitrification with *Fusarium oxysporum* and *Cylindrocarpon tonkinense* (Sutka et al., 2008).

Using isotopomer analysis of N_2O and its substrates, along with other physical and chemical properties of soils, this study was conducted to elucidate the temporal changes of production, consumption, and soil-to-atmosphere emission processes of N_2O in situ from paddies without rice plants during flooding process. We tried to figure out the temporal changes during flooding process using spatial variability of soil water content. We also analyzed the effect of nitrogen fertilizer application on N_2O emissions during that period.

2. Materials and methods

2.1. Study site and field management

Measurements of N_2O emissions and N_2O concentrations of soil gases, in addition to gas sampling for isotopomer analysis, were conducted in rice paddy fields in Tsukubamirai City, Ibaraki Prefecture, Japan (35°58′ N, 139°59′ E). The Fluvisol soil type had the following main soil properties in the plowed layer (to a depth of 13–16 cm): bulk density, 0.87 g cm^{-3}; soil pH (1:2.5 water), 5.9; total C and N contents, 21.4 and 1.97 mg g^{-1}, respectively; percentages of sand, silt and clay, 36%, 40% and 23%, respectively (Hasegawa et al., 2013).

Experiments were conducted five (in 2010) or two (in 2011) days after the beginning of irrigation of 25 April, respectively. In addition, we also made a consecutive monitoring of N_2O flux from 25 April to 9 May, 2011 at one of the control plots. We used five rectangular fields (Fig. 1). The longer side of each field was 100 m and the shorter one ranged from 10 to 70 m. The water source for irrigation was common, and the irrigation water was supplied from one of the shorter side of each field. In 2010, the measurement and sampling was conducted in two rectangular fields (Fig. 1). Each field had four plots. One rectangular field was a control field. Another rectangular field was fertilized. In the fertilized field, blended fertilizer was applied on April 25 at the rate of 6.0 g N m^{-2}, and was incorporated into the soil to a depth of plowed layer (13–16 cm) using a rotary tiller. The blended fertilizer included controlled-