Nitrous oxide emission from grassland and forest soils through nitrification

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The present paper reports laboratory nitrification and nitrous oxide emission in well-aerated soils of a *Miscanthus sinensis* dominated grassland and a *Quercus serrata* dominated warm temperate deciduous forest located at Tokyo University's forest in mid-east Japan. This study analytically appraises the assumptions recognized recently regarding the genesis of nitrous oxide in soil during the nitrification process. The nitrous oxide emission level gently rises in ammonium fertilizer-treated acidic grassland and forest soils (246 and 304 ng/g dry soil) compared to unamended respective soils. This rise in nitrous oxide emission sharply boosts up after lime addition when the pH is raised to 7.4 in the same N-fertilized acidic grassland and forest soils (3451 and 883 ng/g, respective dry soils). These results have direct implications on use of ammoniacal fertilizers to raise the agricultural productivity. Sizable part of the Indian soils is alkaline, well drained, with low moisture. Nitrous oxide emission from such soils during nitrification of fertilizer N may be significant in relation to the potential threat of fertilizer-derived nitrous oxide to the stratospheric ozone layer.

ATMOSPHERIC concentration of nitrous oxide, a greenhouse gas, is increasing at the rate of 0.25 to 0.31% per year[1-4]. Besides being capable of absorbing infrared energy reradiating from the earth's surface, nitrous oxide also participates in the destruction of stratospheric ozone and thus represents a significant pathway for the loss of nitrogen through terrestrial emission[5]. Any increase in the production of nitrous oxide from soils could have a marked effect on the ozone layer, and subsequently on plant and animal life on earth[6,7]. The consensus of opinion is that most of the nitrous oxide produced in soil is by the reduction of nitrate. The denitrification reaction is thought to occur mainly in soils saturated with water or in soils amended with a considerable amount of organic matter[8]. This reaction, and the production of nitrous oxide, is considered to be unimportant in aerobic soils at low moisture contents. Some recent contributions have observed the possibility of nitrous oxide emission from aerobic oxidation of ammonium to nitrite and nitrate nitrogens[9-13]. A large proportion of the earth's land surface contains soils that are intermittently wetted, and are drier than field capacity for most of the time[14]. We, therefore, undertook this study to report nitrous oxide emission from grassland and forest soils in two conditions: (i) natural, well-aerated, low moisture containing nitrifying soils, and (ii) fertilizer N and lime amended soils, in order to appraise the potential contribution by these soils to atmospheric nitrous oxide pool.

Two types of vegetation differing in their ecological status[15] but occupying Andosols (volcanic soils) were selected at Mt. Sekison, Kiyosumi, Chiba prefecture located in mid-east Japan in the Tokyo University's forest area (35°12.5′ N latitude, 40°9′ E longitude at 347.6 m in altitude in Kiwada-bata, Kimitsu City). Out of the two sites, one was *Quercus serrata* dominated successional warm temperate deciduous forest (age about 30 years), and the second was perennial *Miscanthus sinensis* dominated grassland seral stand, resulted due to felling of the deciduous forest as successional plant community, stood 50 m apart from each other and occupying a hilly undulating habitats. Both the soils were slightly acidic (KCl pH 4.2 by 1 : 5 soil : 2 N KCl) with a loamy texture. Other soil characteristics were: 7.22% organic C (by wet digestion method), 0.70% total N (by Kjeldahl), 93.2 µg ammonium N (by KCl extraction and distillation) per g for grassland soil; and 4.95% organic C, 0.53% total N, and 46.0 µg ammonium N per g forest soil, respectively.

An area ranging from 30 × 9 m for each plant communities was selected in the centre of each site, and arbitrarily divided into 30 plots (size 3 × 3 m); ten plots for sampling in winter and summer seasons. A trench of 25 × 25 × 15 cm was dug out after removing the freshly fallen surface litter from each respective season-wise plot of 3 × 3 m. Ten subsamples were collected from each research site representing the entire area randomly, and transported to the laboratory in air-tight vinyl bags at 4° to 5°C in the ice box. Aliquots from field-moist soils collected from each site were pooled to three composite samples and screened through the 2 mm sieve.

For laboratory measurements of mineralization, nitrification and nitrous oxide emission the freshly collected grassland and forest soil samples were further processed and analysed in terms of the following three treatments: (i) natural soils (without any amendment), (ii) natural soils amended with nitrogenous fertilizer (in the form of nitrifiable ammonium sulphate: 200 µg ammonium nitrogen per g soil), and (iii) lime amended N-fertilized soils (calcium carbonate + ammonium sulphate 200 µg ammonium nitrogen per g soil, and KCl pH 7.4). Soil moisture and water-holding capacity were determined gravimetrically in the field-moist and 2 mm sieved soils. Two mm screened fresh soils equivalent to 10 g oven dry weight, after adjusting within 50% to 60% of WHC,

were incubated at 30°C. The incubations were done in 1 litre glass stoppered bottles fitted with a ground glass joint and a stop cock. The entire incubations were planned in triplicate sets. The concentration of evolved nitrous oxide inside the bottle under aerobic conditions was measured at 3, 7, 14 and 21 days intervals by a gas chromatographic technique that uses xenon (Xe) in air as an internal standard[16–19]. This sensitive procedure involves two steps: quantitative trapping of nitrous oxide and Xe from the bottle sample by passing through a Porapak-Q column of the GC cooled to –135°C with using pentene frozen with liquid nitrogen. And subsequently nitrous oxide and Xe are separated by GC (Tracor MT-150G) equipped with a column of Porapak-Q at 65°C and detected by an ultrasonic detector (Tracor U-90). After each determination, the sample bottles were well aerated for a few minutes before again incubation at 30°C. Before and after the 21-days of incubation, aliquots of incubated soil samples were analysed for ammonium (mineralization) and nitrate nitrogen (nitrification) by extraction and distillation method[20]. Such 21 days incubation for nitrous oxide emission and nitrate nitrogen measurement was done season-wise in triplicate sets for natural soils, N-amended soils, and N plus lime amended soils.

The pattern of nitrogen transformation, i.e. nitrogen mineralization (the microbial production of ammonium from organic nitrogen) and nitrification (the microbial oxidation of ammonium to nitrite to nitrate) is striking in grassland and forest soils (Table 1 showing pooled average of both seasons for brevity). During the incubation of 21 days, the mineralization was higher in natural and N amended grasslands as evidenced in stands of lower ecological stage[21]. In contrast to this trend, rate of nitrification increased from lower ecological stage of grassland to the higher one, i.e. in the natural and N amended deciduous forest. These natural and amended soils, with low moisture contents and well-aerated aerobic situations, evolve nitrous oxide. This observation further supports the recent detection and formation of small amounts of nitrous oxide from soils during the formation of nitrite and nitrate through the nitrification process[12,22]. The nitrogenous fertilizer-treated soils in the form of ammonium evolve higher nitrous oxide during the nitrification compared to unamended natural soils (Table 1). This trend has also been reported in well-aerated ammonium sulphate-treated soil with and without the effect of nitrapyrin [2-chloro-6-(trichloromethyl)pyridine][23,24]. This compound inhibits the oxidation of ammonium to nitrite by *Nitrosomonas europaea*, thereby greatly reducing the efflux of nitrous oxide during nitrification.

It is widely believed that acidic soils do not nitrify or do it very poorly[25]. Nitrification and nitrous oxide emission in the forest and grassland soils in the present study proceed well at even lower soil pH (KCl value – 4.2), and nitrous oxide production significantly increased to more than three and ten times respectively at higher pH (slightly alkaline) when amended with lime. The study indicates that nitrous oxide production via nitrification in well-aerated soils with ammonium-yielding fertilizers has enormous emission potential at higher pH level in soils and thus significant source of important greenhouse trace gas. The decreasing trend in net nitrogen mineralization due to N-immobilization in natural, fertilizer-added, and fertilizer plus lime-added soils under the investigation, and increasing trend in nitrification indices closely reflect to pattern in nitrous oxide effluxes (Table 1). Across the range of grassland and forest soils examined, the linear correlation coefficient for nitrification versus nitrous oxide flux is 0.892 (significant at $p < 0.01$, $n = 36$).

Table 1. Nitrogen mineralization, nitrification and nitrous oxide emission for natural, N fertilizer and lime amended well-aerated grassland and forest soils*

Soil	Mineralization (µg N/g)	Nitrification (µg N/g)	Nitrous oxide flux (ng/g)
Natural grassland	13.5 (9.5)	32.5 (4.5)	207 (19)
N-added grassland	6.5 (1.5)	32.0 (1.0)	246 (33)
N and lime-added grassland	–144.0 (51.0)	352.0 (84.0)	3541 (204)
Natural forest	9.0 (1.0)	43.5 (8.5)	279 (16)
N-added forest	0.5 (0.1)	44.5 (7.5)	304 (9)
N and lime-added forest	–144.5 (21.5)	232.5 (70.5)	883 (166)

*Field-moist soils were incubated (60% WHC) for 21 days at 30°C after treatments specified in the text. Values in brackets are standard error. For brevity, data are pooled to average for both the seasons.

The quantity of work described in this paper is limited. Nevertheless, the higher emission of nitrous oxide in alkaline forest and grassland soils treated with N-fertilizer is significant for potential threat to stratospheric ozone. This observation may provide a base to work out, hitherto unexplored, the nitrifying source of nitrous oxide emission from well-aerated and drained Indian soils largely having alkaline pH and N-fertilizer application to boost the agricultural production. As recently the fertile Arabian sea in the Indian Ocean was reported as chimney of nitrous oxide under the denitrification[26,27].

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Moisture desorption and absorption isotherms for seeds of some cultivars of *Triticum dicoccum* wheat

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Moisture desorption and absorption isotherms for 50 seeds each of thirty varieties of *Triticum dicoccum* wheat in five replications were recorded at 30°C and 85% RH. Hysteresis loops were established for all the varieties from the average seed masses, normalized to equal curve heights for meaningful comparison. It is observed that the shapes and area enclosed by hysteresis loops for seeds of different varieties are different. Curiously enough, the seeds of varieties with smaller area under their moisture hysteresis loops hold more per cent initial water in them at initial saturation at 30°C and 85% RH, than the seeds of varieties with large area under hysteresis loops. Whereas the behaviour of tetraploid *Triticum durum* and *Triticum dicoccum* wheat varieties are parallel, the behaviour of hexaploid *Triticum aestivum* varieties is considerably different in respect of moisture absorption. Typical examples of the dynamics of moisture movements in seeds of two *Triticum dicoccum* varieties are presented and discussed. It is believed that the observations discussed in this paper would be of considerable help to wheat breeders for improving the *dicoccum* wheat for yield, particularly in the rainfed areas of Karnataka, Maharashtra and Gujarat, where varieties of this species are still being grown on commercial scales for some specific end-products.

MOHARIR[1], and Moharir and Nam Prakash[2] recently studied the moisture desorption and absorption isotherms, for seeds of some well-known cultivars of *Triticum aestivum* and *Triticum durum* wheats, at 30°C and 85% RH, and established moisture hysteresis loops from the average normalized masses of 50 seeds of each variety, during the dehydration and rehydration cycles. It has been shown[1,2] that not only the shapes of these hysteresis loops are different for different varieties, but also the area enclosed under them are different. Curiously enough, the area enclosed under the hysteresis loops for seeds of well-established rainfed cultivars of *T. aestivum* (bread wheat) is smaller than that for the seeds of well established irrigated varieties. Similar observations were also recorded for seeds of *T. durum* wheat varieties[1,2] as well. However, the range of variation in hysteresis loop area for seeds of *durum* varieties is considerably smaller[2]. This has been attributed to relatively shorter breeding history for *durum* wheat[2,3]. Further, the rates of absorption and desorption of water, for seeds of rainfed varieties, were slower as compared to those for the seeds of