J. KOREAN SOC. SOIL SCI. FERT. 1983. Vol 16 No. 4

A Study of Denitrification in the Grassland with an Acetylene Inhibition Technic

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草地에서의 아세칠렌 阻害法에 依한 脫窒 硏究

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摘 要

本 実験은 20個所의 草地土壤에서 土壤을 column으로 採取하여 脫窒系에 아세틸렌 가스를 注入함으로서 모든 脫窒最終生成物을 N₂O로 誘導하는 方法으로 草地에서의 脫窒을 測定하였던 바 그 結果는 다음과 같다.

1. 脫窒系에 C_2H_2를 注入하지 않았을 경우, 대부분의 試驗区에서 N_2O의 発生은 探知되지 않았으나, C_2H_2를 注入함으로서 모든 脫窒生成物은 N_2O로 探知되었다.

2. 脫窒系를 물로 포화시켰을 때는 거의 모든 区에서 脫窒이 発生했으나, 自然状態의 土壤column에서는 거의 脫窒이 일어나지 않았다.

3. 尿素를 施肥한 후 13日째로부터 16日째 사이의 3日間 土壤column을 培養했을 경우 各 区의 平均 脫窒量은 4.2%였으며, 最大는 14.2%였다.

4. 脫窒과 脫窒系土壤의 化学的性質과의 사이에는 거의 相関이 없는 것으로 나타났으나 이는 土壤의 化学性을 培養이 끝난 후에 행했기 때문인 것으로 判断했다.

Introduction

Nitrogen loss from crop field occurs through the removal of harvesting plants, surface run-off, leaching through soil profile, volatilization as ammonia or nitrite and denitrification. Denitrification is a process of reduction of nitrate or nitrite to nitrous oxide or molecular nitrogen, in which molecular nitrogen is more common. Nitric oxide is also one of the products of denitrification but it is readily oxidized as NO_2 in the presence of oxygen.

Recently attention has been paid to denitrification because of the increased cost of fixed nitrogen and the possibility that denitrified nitrous oxide may contribute to atmospheric ozone depletion in the stratosphere.[7)]

Nitrogen loss by denitrification has traditionally been determined from the balance sheet in N budget, since no method for its reliable and routine field determination has been available. The reliability of values obtained by difference, however, are lower than that of the other measurements, and all errors are accumulated in the difference value. And it is not always easy to obtain accurate measurement of leaching, plant uptake, and run-off especially in the field.

Ammonia and nitrite gas volatilization loss is another factor to be misunderstood as denitrification in the balance sheet analysis.

In direct measurement of denitrification, analysis of denitrified molecular nitrogen is difficult because N_2 is omnipresent in most systems even when the air N_2 is replaced with an inert gas such as He or Ar. ^{15}N-labelled compounds are also used in direct measurement of denitrification but it involves mass spectrometric analysis and is relatively insensitive.

As mentioned above, nitrous oxide is one of the free products of denitrified nitrogen. The proportion of nitrous oxide evolution during denitrification in soil systems has been reported to be from

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0 to 100% of the total gases produced.[5] If nitrous oxide is the sole denitrification product, analysis is greatly simplified since nitrous oxide is a minor atmospheric constituent and can be analyzed accurately by gas chromatographic detectors.

Recently Federova et al reported that acetylene inhibited the reduction of nitrous oxide to molecular nitrogen during denitrification.[5] It was confirmed by pure culture studies of denitrifying bacteria.[2] Consequently, it became available to detect the total amount of denitrified nitrogen from soil as nitrous oxide.

It has been elucidated that denitrification occurs vigorously at submerged soils such as paddy fields having lower oxygen partial pressure and containing much organic matter readily decomposable.[1] Grasslands are distributed at wide ranges of soil status and accumulate a large amount of organic matter such as roots of dead plants and litter by fallen leaves and defoliated residues locally. Therefore, grassland has a possibility to induce a transient denitrification with the development of anaerobic conditions by rainfall or carbon dioxide gas evolved from microbial respiration. However, little denitrification study in the grassland has been available.

This experiment was carried out to detect denitrification in the grassland and to test the possibility of the application of acetylene inhibition technic, the inhibition of nitrous oxide reduction to dinitrogen under the undisturbed soil condition.

Materials and Methods

1. Selection of Experimental Sites and Analysis of N_2O gas

Twenty experimental plots of 1 m^2 size were selected from various grasslands as shown in Table 1. Urea as nitrogen fertilizer was applied at the rate of 200kg/ha for each plot. Thirteen days after fertilizer application, soil was taken from each plot by an iron column (ϕ10.5x15cm). The iron columns with soil were watered at the maximum water holding capacity and were placed in each polyethylene bag. The polyethylene bags prepared were introduced with 0.9 atm(v/v) of air and 0.1 atm of acetylene gas. After a three-day incubation at room temperature of 25-30°C, a portion of aerial part was taken with a gas tight syringe and nitrous oxide evolved was analysed by a gas chromatography in a routine method.[10]

2. Analysis of soil properties

Soils after incubation were air-dried and prepared for the analysis of soil properties.

Soil pH, total carbon(T-C), total nitrogen(T-N), NO_3-N and NO_2-N: Soil samples passed through a 2 mm sieve were used for the respective analysis with the following methods.

soil pH : pH meter(HORIBA H-7mv),
soil : H_2O=1 : 2.5

T-C and T-N : C/N corder(YANACO MT-500)

NO_3-N : colorimetric method(color developed by phenolsulfuric acid and ammonia water)[9]

NO_2-N : colorimetric method(reacted with sulfanilamide and color developed with N-1-naphtylethylenediamine hydrochloride)[9] Soil mineralizable carbon : Soil mineralizable carbon was estimated by determining the amount of carbon evolved as CO_2 on incubation of 20 g of soil with saturating to the maximum water holding capacity at 30°C for 3 days.[3] Incubation was performed in 100 ml Erlenmeyer flasks sealed with serum caps, and CO_2 evolved was determined by a gas chromatography.[10]

Results and Discussion

Table 1 shows the estimation of N_2O evolved per day under watered conditions. In most of the soils examined, N_2O was not detected or negligible when C_2H_2 was not introduced into the system, indicating that introduced C_2H_2 blocked the pathway, N_2O to N_2. N_2 was not detected as a denitrification product in a separate model experiment where atmospheric N_2 was replaced with He. Consequently, C_2H_2 inhibition technic can be applicable in the field study of denitrification. Details of this technic are reported elsewhere.[6]

Generally, ammonification and nitrification from urea in the soil take place rapidly in the adequate

Table 1. Denitrification as N_2O from soil columns with the introduction of C_2H_2

plot	denitrification as N_2O (μM/column/day)	plant	coverage (%)
1	5.1	young grass unidentified	10
2	63.2	young grass unidentified	10
3	40.7	bahiagrass	90
4	15.3	no plant	0
5	111.9	Italian rye grass	80
6	71.2	ladino clover	80
7	56.0	ladino clover	80
8	10.2	no plant	< 5
9	0	young grass unidentified	10
10	0	orchard grass	20
11	20.3	no plant	0
12	152.5	no plant	0
13	111.9	orchard grass	90
14	20.3	no plant	0
15	35.6	no plant	0
16	25.4	Italian rye grass	10
17	15.3	no plant	0
18	45.8	no plant	0
19	56.0	alfalfa	10
20	30.5	alfalfa	10
mean	44.36±41.22		

period was high enough for denitrifying process in this experiment, and the nitrification of urea did not seem to restrict denitrification.

Soil nitrate nitrogen contents before the setting of incubation for denitrification were not determined. If original soil nitrate and nitrified nitrate during the incubation were neglected, 4.2% in average and 14.2% in maximum of applied urea were assumed to be denitrified as N_2O during the 3-day incubation period. In the present experiment, diffusion of N_2O evolved in the closed system might be restricted more or less. This would result in the lower values of denitrification than the values in the natural system where gas diffusion is free. In addition, since the denitrification loss of 4.2% in average is the result of only 3-day incubation, more denitrification is anticipated before and after incubation. Accordingly, denitrification seems to occur even in the grassland when soil is saturated with enough water by rainfall. In the pre-experiment, without soil saturation with water, denitrification was nil or neglible.

Denitfification is known to be affected not only by soil environmental factors but also by soil properties.[1] Soil properties examined after the incubation experiment are shown in Table 2. Slight alkaline soils(pH 7-8) are generally recognized to favor denitrification.[4, 8] The result of this experiment, however, did not agree with general recognition. The pH of soil used here ranged from 5.4 to 7.4, and did not correlate to denitrification(r=0.049). The denitrifying bacteria are essentially aerobic except for the ability to utilize nitrate or nitrite in the absence of oxygen. Nitrate and nitrite act in lieu of oxygen as a terminal acceptor of electrons produced during

Table 2. Soil properties of the columns

Plot	Soil pH (H_2O) 1 : 2.5	NO_3-N μg/100g (dry soil)	NO_2-N μg/100g (dry soil)	T−C (%)	T−N (%)	C / N	Mineralizable C mg/flask/day
1	5.5	98.7	15.0	0.98	0.08	12.6	0.94
2	6.4	503.3	15.0	1.56	0.14	11.4	1.17
3	6.1	2956.8	25.0	0.95	0.08	12.2	0.84
4	5.4	135.4	60.0	0.89	0.08	11.4	0.68
5	6.5	125.0	25.0	0.73	0.07	10.4	1.00
6	6.0	261.3	25.0	1.08	0.09	11.5	1.27
7	6.9	0.0	787.5	2.16	0.17	12.6	2.12
8	6.0	1020.2	52.5	1.33	0.10	13.1	0.72
9	6.0	279.1	30.0	1.68	0.13	12.7	1.08
10	6.1	197.2	30.0	0.95	0.07	13.6	0.78
11	6.2	792.7	30.0	1.30	0.12	10.8	1.02
12	6.5	376.2	37.5	1.11	0.09	11.9	0.75
13	5.6	129.1	30.0	0.76	0.05	16.2	0.42
14	6.9	0.0	2017.5	2.16	0.20	10.6	1.23
15	6.9	1382.2	45.0	1.43	0.23	11.1	0.58
16	7.3	2639.3	42.5	1.65	0.15	11.1	1.03
17	7.0	5068.1	45.0	0.70	0.07	10.6	0.36
18	7.3	540.9	500.0	1.46	0.13	11.0	0.99
19	7.4	1395.9	45.0	1.65	0.14	11.7	0.87
20	7.3	1439.8	37.5	1.08	0.08	13.2	1.09

anaerobic respiration. In this experiment, soils were saturated with water and consequently the supply of oxygen was limited and nitrate or nitrite would act as an electron acceptor. Soil nitrate and nitrite contents were not correlated to denitrification in this experiment(r=-0.028 and -0.08, respectively) and rather showed slightly negative correlation. This can be explained that the nitrate and nitrite present in the soil before incubation might have been exhausted during the incubation.

It is natural that total nitrogen did not show significant relationship with denitrification(r=-0.169) because most portion of total nitrogen would be contained in organic matter and organic matter decomposition would not occur so rapidly during the 3-day incubation.

Other soil chemical properties such as soil total carbon, C/N ratios and mineralizable carbon did not correlate to denitrification, either(r=-0.177, 0.138, and 0.091, respectively). Organic matter will serve as the energy source for the heterotrophs of denitrifying bacteria and there should be some relation between T-C(raw organic matter and litter were excluded from T-C) or mineralizable carbon and denitrification. This can be explained with the same ground as soil NO_3-N and NO_2-N. Denitrified nitrogen was measured for the undisturbed core soil and soil chemical properties were done for the disturbed soil after denitrification measurement. Accordingly, if soil chemical properties were determined before incubation though impossible for the same undisturbed soil, there may be some relationships between soil properties and denitrification. From the result of this experiment, an elaborate laboratory experiment to clear the relation between soil properties and denitrification is necessitated since field studies for denitrification should comprise undisturbed soil system.

Abstract

A field study was conducted for 20 soil columns to investigate the denitrification in the grassland with a method introducing C_2H_2 in the denitrifying system. Since acetylene blocked the pathway from N_2O to N_2, all the free products of denitrification consisted of nitrous oxide. In this study, denitrification was measured as N_2O. Results are as follows.

1. In most of soils examined, denitrification was observed as N_2O when acetylene was introduced into the denitrification system while N_2O was scarcely evolved without acetylene in the system.
2. Denitrification occurred even in the grassland soils when they were saturated with water. Denitrification was nil or negligible without water saturation.
3. Denitrification loss for 3 days incubation(from 13th to 16th day after urea application) was 4.2 % in average and 14.2 % in maximum.
4. Soil chemical properties such as nitrate nitrogen, nitrite nitrogen, total nitrogen, total carbon and mineralizable carbon did not show clear relationships with denitrification, probably since soil chemical properties were analysed for the soils after incubation.

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