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学位論文題名

Effect of soil aggregate on nitrous oxide production from different soils

（異なる土壌における亜酸化窒素の生成に対する土壌構造の影響）

学位論文内容の要旨

Introduction

In the last few decades there has been an increase in the emission of greenhouse gases (GHGs, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Soil is an important source and sink of GHGs. Properties and environmental factors of soil such as moisture content, temperature, pH, organic matter, O_2 status and the capacity of soil to supply available N, influence N_2O release from soil. Soil aggregates play an important role in both water retentivity and permeability, which influence biological processes. Managed grasslands are major contributors to the biosphere–atmosphere exchange of these three gases, with fluxes closely linked to management practices and soil types. Moreover, manure application increase GHGs especially N_2O has also been reported. Therefore, the present study of laboratory incubation was carried out to determine the effect of aggregate size, moisture content, fertilizer management on N_2O and CO_2 production in three grassland soils with different texture.

Materials and Methods

Soil samples were taken from the mineral soil layer (just below the root mat) in the thickness of about 10 cm in three grasslands of Shizunai, Shintoku and Hamatonbetsu. Shizunai soil was a volcanogeneous sandy soil and was sampled from both chemical fertilizer and manure applied fields. Shintoku soil was a volcanogeneous light clay soil and was sampled from mainly chemical fertilizer applied field. Hamatonbetsu soil was a grey upland clay loam soil and was sampled from also mainly chemical fertilizer applied field. After air dry of those soil samples, they were gently ground by hand to pass through a sieve, aggregate samples with the sizes of 2mm and 4.5mm were prepared. A 20 g dry basis soil sample, after adjusting the soil moisture content of 60% and 80% of field water capacity (FWC), was loosely packed in an 80 ml plastic cup into a 1.8 L of Mason jar. The jar was sealed tightly and incubated at 20 °C for 24 hours, Air samples were taken from the head space of the jar and concentrations of CO_2, N_2O and nitric oxide (NO) were analyzed by an infra-red CO_2 analyzer, a gas chromatograph with ECD and NOx analyzer, respectively. NO was measured, because N_2O-N/NO-N is an indicator of nitrification and denitrification. Production rate of each gas was calculated from the difference between initial and final gas concentrations in the 24 hour incubation. Initial gas concentration was measured just before incubation using a blank jar. Incubation was conducted for 9 days at three replicates. Before and after incubation, the concentrations of water extractable organic carbon (WEOC), NH_4^+-N and NO_3^--N and soil pH, total C and total N were analyzed. Soil microbial biomass C (MBC) before incubation and potential denitrification enzyme activity (DEA) after incubation were also analyzed. All analyses were conducted with three replicates. N mineralization and WEOC consumption were estimated from the difference of concentrations of mineral N and WEOC between before and after incubation, respectively.

Results and Discussion

1) In all samples, chemical properties, especially WEOC and NH_4^+-N, changed immediately after adding the water, and this situation continued throughout the incubation process. Following the start of incubation, a flush of N_2O was observed, and similar flushes of CO_2 and NO were observed. Coarse textured Shizunai soil showed larger change in chemical properties and the flushes of the gases compared to the fine textured Shintoku and Hamatonbetsu soils.

2) Larger aggregates of all soils showed higher values of chemical properties, especially WEOC, MBC, NH_4^+-N and NO_3^--N, and higher productions of N_2O, CO_2 and NO compared to smaller aggregates. The higher amount of MBC in larger aggregates induced higher decomposition of organic matter, resulting in greater N_2O, CO_2 and NO productions, WEOC consumption and N mineralization. .

3) There was a tendency that soils in higher moisture content showed much higher chemical properties and much higher gases productions. MBC and DEA were enhanced by higher moisture, leading increase of WEOC consumption. There was no significant difference in N mineralization between moisture contents in fine textured soils, while in coarse aggregated soils, higher soil moisture stimulated N mineralization significantly.

4) Increase of NO_3^- -N content after incubation indicates that the consumption of NO_3^- -N by denitrification was slower than the production of NO_3^- -N from nitrification. Comparing among the chemical fertilizer applied soils, NH_4^+-N increased in all the soils, but the tendency of change in NO_3^--N was different among three soils. Regardless of soil moisture, NO_3^- -N decreased in coarse textured Shizunai soil, while NO_3^- -N increased in fine textured Hmatonbetsu soil. In case of finest textured Shintoku soil, NO_3^--N decreased in 60% of FWC, but NO_3^- -N increased in 80% of FWC. It seemed that increase of moisture content stimulated nitrification in fine textured soil.

5) Manure application stimulated N_2O, CO_2 and NO productions, WEOC consumption and N mineralization significantly. In the manure applied Shizunai soil, NH_4^+-N and NO_3^- -N increased during the incubation, while NH_4^+-N increased but NO_3^- -N decreased in the chemical fertilizer applied soil. Manure application stimulated nitrification, leading more denitrification.

6) Microbial biomass carbon was higher in fine textured soil. Coarse textured Shizunai soil showed that MBC correlated with N mineralization, WEOC consumption and DEA significantly. This suggests that the suitable aeration of this coarse textured soil enhanced organic matter decomposition, and the soil was able to quickly develop the condition suitable for denitrification after moistening. These led that the soil produced more N_2O immediately compared to other fine textured soils. On the other hand, in fine textured Shintoku and Hamatonbetsu soils, MBC did not correlated with N mineralization, although MBC correlated with WEOC consumption and DEA significantly. This suggests that the fine textured soils could not produce enough NO_3^--N for denitrification due to poor aeration of the soils in spite of high content of MBC. Furthermore, fine textured soils made the diffusion speed of NO_3^- -N slow. Those reduced N_2O production.

7) Fine textured soils and coarse textured soil with lower soil moisture showed that CO_2 production was greater than WEOC consumption. This indicated that WEOC was consumed by mainly decomposition. The coarse textured soil in 80 % of FWC showed greater WEOC consumption than CO_2 production and greater aggregate size stimulated it. This indicated that WEOC was consumed not only by decomposition but also by assimilation. These findings suggest that microbial activity and biomass increased in the condition of well aeration and higher moisture, leading well N transformation but high N_2O production.

8) The process (nitrification/ denitrification) of N_2O production during incubation were different among these soils. It is well known that N_2O-N/NO-N ratio is less than 1 in nitrification, while the ratio is greater than 100 in denitrification. In fine textured Shintoku and Hamatonbetsu soil, N_2O-N/NO-N ratio mostly ranged between 0.01-1 in 60% of FWC and 1- 100 in 80 % of FWC and the N_2O production rate increased with an increase of N_2O-N/NO-N ratio from 0.01 to 1 in 60% of FWC and with a decrease of the ratio from 100 to 1. These findings indicated that the N_2O production was derived from mainly nitrification. On the other hand, in the coarse textured Shizunai, N_2O-N/NO-N ratio ranged between 1-100 in both 60% and 80 % of FWC. However, the relationship between the ratio and N_2O production rate was different. In the 60 % of FWC, the N_2O production rate increased with a decrease of N_2O-N/NO-N ratio, indication the process was mainly nitrification. In the 80 % of FWC, the N_2O production rate increased with an increase of N_2O-N/NO-N ratio, indicating the process was mainly denitrification.

Conclusion

This study revealed that 1)Larger aggregate and higher moisture content produced more N_2O, CO_2 and NO production; 2) Moistening induced strong effect on the flushes of the gases; 3) Soil texture determining pore size distribution was significant controlling factor for gases productions; which regulates aeration (O_2 diffusion), affecting microbial biomass and activities as organic matter decomposition, nitrification and denitrification; 4) Coarse textured soil with high moisture produced more N_2O through denitrification compared to fine textured soil, however, it produced more mineral N, CO_2 and consumed more WEOC; 5) Larger aggregate and manure application enhanced them.

学位論文審査の要旨

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学位論文題名

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（異なる土壌における亜酸化窒素の生成に対する土壌構造の影響）

本論文は英文121頁，図47，表15，5章からなり，参考論文1編が付されている．最近の数十年間，温室効果ガス（GHGs；二酸化炭素（CO_2），メタン（CH_4），亜酸化窒素（N_2O））の放出は増加し続けている。土壌は，GHGsの重要なソースおよびシンクである。水分，地温，pH，有機物，酸素状態，供給された利用可能窒素に対する容量などの土壌の特性や環境要因は，土壌からのN_2O放出に影響を与える。土壌団粒は，水分保持と透水性の両方において重要な役割を担い，生物学的プロセスに影響を与える。さらに，堆肥の施与はGHGs放出，特にN_2O放出を促進することが報告されている。そのため，団粒サイズ，土壌水分，施肥処理がN_2OとCO_2生成に与える影響を明らかにするため，土性の異なる土壌を用いて培養実験をおこなった。

土壌試料は，静内，新得，浜頓別の3地区の草地においてルートマット直下の無機質土壌層(約10cm)から採取した。静内は火山灰由来の粗粒な砂質土で，化学肥料で管理された圃場と堆肥で管理された圃場の両方から採取した。新得は，火山灰由来の細粒な軽埴土，浜頓別は，灰色台地土の細粒な埴壌土で，これらは，主に化学肥料で管理された圃場から採取した。土壌試料は風乾後，手で慎重に細かくしながらふるいを通し，2 mmと4.5 mmの団粒を調整した。各土壌団粒の圃場容水量（FWC）の60%と80%に土壌水分を調整したあと，乾土重20 gの試料を80 mLのプラスチック製容器に緩く詰め，1.8 Lのガラス瓶に入れた。瓶を密閉後，20℃で24時間培養し，瓶のヘッドスペースから採取したガス試料のCO_2，N_2Oおよび一酸化窒素（NO）濃度をそれぞれ赤外線ガス分析計，ECD付きガスクロマトグラフ，NOx分析計で測定した。NOを測定したのは，N_2O-N/NO-N比が硝化と脱窒の指標となるからである。各種ガスの生成速度は，培養24時間における最初と最後のガス濃度から算出した。初期濃度は，培養開始時におけるブランク瓶の濃度とした。培養は9日間，3反復でおこなった。培養の前後で，水抽出性有機態炭素（WEOC），NH_4^+-N，NO_3^-N，土壌pH，全窒素および全炭素を測定した。培養前の土壌微生物バイオマス（MBC）と培養後の脱窒活性（DEA）もそれぞれ3反復で測定した。窒素無機化とWEOC消費は，それぞれ培養前後の無機態窒素濃度，WEOC濃度の変化量から求めた。

全試料で，化学性，特にWEOCとNH_4^+-Nは水添加後直ちに変化し，それは培養期間中持続した。培養の初めにN_2Oのフラッシュが観測され，CO_2とNOにも同様のフラッシュ

が生じた。粗粒な静内土壌では，細粒な新得と浜頓別土壌に比べ，より大きな化学性の変化とガスのフラッシュが生じた。

全ての土壌で，大きな団粒で，N_2O，CO_2，NO生成量は大きく，WEOCとMBC，NH_4^+-N，NO_3^--Nは高い値を示した。高い土壌水分で，ガス生成量は大きく，化学性も高い値を示した。堆肥施与は，ガス生成量を有意に増加させた。

培養後にNO_3^--N濃度が上昇する場合と低下する場合が認められた。NO_3^--N濃度が上昇する場合は，脱窒によるNO_3^--N消費が硝化によるNO_3^--N生成を下回っていたことを示す。このことを化学肥料施与区の結果で土性の違いを比較すると，NH_4^+-Nは全ての土壌で上昇したが，NO_3^--N変化量は土壌によって異なった。土壌水分に関わらず，粗粒な静内土ではNO_3^--Nは減少したが，細粒な浜頓別土ではNO_3^--Nは増加し，細粒な新得土では，FWC60%でNO_3^--Nは減少，FWC80%でNO_3^--Nは増加した。細粒土では，土壌水分の上昇が硝化を促進すると考えられた。なお，N_2O-N/NO-N比が1以下では硝化，100以上では脱窒が優勢であることがよく知られている。この関係から見ても，N_2O生成は，細粒な新得と浜頓別ではいずれの条件でも硝化由来であったが，粗粒な静内土では，脱窒に由来することが示された。

粗粒な静内土では，MBCは窒素無機化量，WEOC消費量，DEAと有意な相関を示した。一方，細粒な新得と浜頓別土では，MBCはWEOC消費量とDEAと有意な相関を示したが，窒素無機化量とは関係が認められなかった。これらのことは，土壌は水添加後直ちに有機物分解と窒素の無機化および脱窒に適した状態を発達させるが，粗粒土では最適な通気性が硝化を促進させ脱窒に十分なNO_3^--Nを生成するのに対し，細粒土では通気性が悪く脱窒に十分なNO_3^--Nを生成できなかったことを示している。

本研究で明らかになったことは次の通りである。1）大きな団粒サイズと高い土壌水分は，N_2O，CO_2，NO生成を促進する。2）水添加は，ガスのフラッシュを強く誘発する。3）土性は，ガス生成を制御する重要な要因であり，通気性を制御することで土壌有機物分解，硝化，脱窒など微生物活性およびバイオマスに影響を与える。4）高い土壌水分の粗粒土では，細粒土に比べると，脱窒によりN_2O生成が促進される。5）大きな団粒サイズと堆肥施与でそれらはより促進される。

以上のように，本研究は，土壌団粒が土壌中の温室効果ガス生成に強く影響を及ぼすことを示したものであり，土壌からの温室効果ガス発生の見積もり精度の向上に新しい知見を与えるものである。よって審査員一同は，Farzana Diba が博士（農学）の学位を受けるのに十分な資格を有するものと認めた.