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Microbial Biomass in Soils as Influenced by Different Nutrients

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Abstract An incubation experiment was conducted to evaluate the effects of several mineral nutrients application on the soil microbial biomass formation in a granitic regosol of Japan which have very low chemical fertility. Several nutrients (N, P, K, Ca, Mg and S) application treatment was assigned as a control plot and each nutrient reduction treatment was considered as (−N), (−P), (−K), (−Ca), (−Mg) and (−S) plot. These mineral nutrients of each plot were applied as the nutrient solution with 1000 mg C kg^{-1} soil (glucose) to the regosol and the amount of microbial biomass was compared with the control. The amount of microbial biomass C markedly increased with each nutrient application, and the maximum values of microbial biomass of each plot were significantly different among the nutrients applied. While, the specific respiration of microbial biomass was decreased with increasing microbial biomass C. Relative values of microbial biomass C of each plot over the control was quite similar at 5 days and 10 days after nutrients application, it was the lowest at −N plot (52–55%) followed by −P (62–65%), −S (67–72%), −Ca (74–81%), −K (80–91%) and −Mg (91–96%) plot, respectively. From these results, it was concluded that not only C and N but also P, S and Ca application was essentially required for microbial biomass formation in the granitic regosol of Japan.

Key words: soil microbial biomass, regosol, mineral nutrients, specific respiration,

INTRODUCTION

Regosols which are widely distributed in Japanese arable land have a coarse texture, extremely low CEC, base saturation, organic matter and available N, P and S. Therefore, nitrogen (N) and phosphorus (P) application are generally required for plant production on a granitic regosol. It is important to generate the microbial biomass to improve plant production. Plant and soil responses to added nutrient are of particular interest in understanding the potential effect of nutrient application on soil ecosystems. Evaluation of nutrient limitation in soil is usually focused on energy or macro nutrient supplies. If the soil microbes use the soil amendment, a variety of responses in the microbial biomass may be observed (Scheu and Parkinson, 1995).

The amount of microbial biomass was affected by soil chemical condition and the recovery of P applied were considerably increased by the organic matter application (Kouno et al., 1994). The microbial biomass formation was significantly correlated with soil C (Marumoto, 1984), and C resources generally control the soil microbial biomass (Anderson

and Domsch, 1985; Sparling et al., 1985). Furthermore, the microbial biomass was also significantly correlated with mineral N amendment (Biederbeck et al., 1984; Jenkinson, 1990). Although, relationships between microbial biomass formation and soil organic C and N are well understood, less is known about the influence of other elements e. g. P, S, Ca, K and Mg. Incubation experiment was therefore conducted to evaluate the effects of several mineral nutrients application on soil microbial biomass formation in regosol of Japan.

MATERIALS AND METHODS

Soils

A granitic regosol (regosol) was collected from the experimental field of Hiroshima university. Chemical and physical properties of the soil are presented in Table 1. The soil samples were collected from the upper 0–20 cm of the profile and hand picked to remove large pieces of plant material. The soil was then sieved (<2mm) and adjusted to 40% of water holding capacity (WHC) with distilled water, and kept moist under aerobic conditions at 25°C for 10 days in a sealed drum containing soda-lime, to allow respiration to settle down after sampling. After this initial conditioning, the soil was used immediately for incubation.

Treatments and experimental design

To optimize the nutrient condition in soil for microbial biomass formation, several nutrients (N, P, S, Ca, K and Mg) were applied to the soil, and assigned as a control plot. Each nutrient reduction treatment from the control was considered as (−N), (−P), (−K), (−Ca), (−Mg) and (−S) plot. These nutrients were applied as a nutrient solution. The nutrient solution were prepared by dissolving the appropriate amount of 1000 mg C (glucose), 300 mg N[NH_4Cl or $(NH4)_2SO_4$], 200 mg P[KH_2PO_4 or NaH_2PO_4]; 60 mg K[K_2SO_4 or KCl]; 100 mg Ca, [$CaCl_2\ 2H_2O$]; 40 mg Mg[$MgSO_4\ 7H_2O$ or $MgCl_2\ 6H_2O$] and 20 mg S[$(NH_4)_2SO_4$ or Na_2SO_4]kg^{-1} soil. Each nutrient was prepared to be equal to the control by using different chemical form. Soil without nutrient application was also prepared, and assigned as original. The soil samples of each plot were re-adjusted to 40% of WHC and each weighed into 100 ml glass bottle. The bottles were then placed in 1 L jar and incubated in the dark at 25°C for 10 days. To prevent drying of soil samples, 10 ml of distilled water was added to each jar. To trap CO_2 evolved by soil microorganisms during incubation, 20 ml of 1M NaOH solution in a 30 ml vial was also placed inside each jar. Soil samples were divided into two portions, one was used for microbial biomass analysis and another portion was air dried and used for chemical analysis. Microbial biomass C and microbial specific respiration were measured at 5 days and 10 days after nutrients application.

Table 1 Characteristics of the soil used

Soil used	Organic C	Organic N	Total P	Extractable P		Exchangeable cations			Available S
	mg kg^{-1} soil			mg kg^{-1} soil		mg kg^{-1} soil			mg kg^{-1} soil
				Troug	BrayII	K	Ca	Mg	
Regosol	trace	trace	84	1.6	1.8	78	30	12	2.5

Microbial biomass measurement

Microbial biomass C was determined by fumigation extraction (FE) method (Wu et al. 1990). Moist soil samples containing 20 g dry soil of regosol was fumigated with ethanol free $CHCl_3$ for 24 h. After $CHCl_3$ removal, the soil samples were extracted with 80 ml of 0.5 M K_2SO_4 for 30 minutes. At the same time, non fumigated soils were also extracted under the same conditions. The extracts were first acidified to pH 2 with HCl and bubbled by N_2 gas to eliminate the inorganic C before organic C determination. The organic C extracted was analyzed by a total organic carbon analyzer (Shimadzu TOC-5000). Microbial biomass C was estimated from the increase in K_2SO_4-extractable organic C (E_C) after fumigation, where E_C is the difference between organic C extracted in fumigated samples and organic C extracted in the non-fumigated samples. Microbial C (B_C) was estimated from the equation; $B_C = 2.22 \times E_C$.

Microbial specific respiration

Microbial specific respiration was measured as CO_2 evolution from soil samples which was trapped in NaOH. During subsequent incubation, NaOH was renewed every 5 days. Total CO_2 was then titrated with 0.4 M HCl using an automatic titrator. The microbial specific respiration was expressed as mg CO_2-C evolved per unit microbial biomass C (mg mic. C), and unit time (day).

Soil chemical and physical analyses

Total C and total N of the air dried samples were determined using a carbon nitrogen analyzer (Yanaco C-N Corder MT 500). Total P in soil was extracted by H_2SO_4 and HNO_3 (1:1, v/v) double acid digestion and determined colorimetrically by the ammonium-molybdate ascorbic acid method (Murphy and Riley, 1962). Available P was determined in aliquots of 0.5 M $NaHCO_3$ (pH 8.5) by the ammonium molybdate blue method (Olsen et al., 1954). Available S was extracted with 0.01M $Ca(H_2PO_4)_2$ and determined by ion chromatographic analyzer. Soil pH was measured using a glass electrode (1:2.5 soil-N KCl).

Tabel 2 Microbial biomass at 5 and 10 days after nutrients application

Treatments	Microbial biomass C (mg C kg^{-1} soil)	
	5 days	10 days
Original	58.9	54.1
Control	413.7	153.5
−N	230.8	81.1
−P	259.3	100.9
−S	281.8	110.8
−Ca	307.8	125.3
−K	333.9	139.4
−Mg	378.9	148.1

Control: Soil with complete nutrient application
Original: Soil without nutrient
− : Nutrient reduced

Statistical analyses

All results are expressed as a mean of three replicate determinations on an oven dry (105°C, 24h) soil basis. A one-way ANOVA was performed to determine the significant differences among the treatments, followed by test on LSD. The LSD was used to compare the nutrient effect on microbial C.

RESULTS AND DISCUSSION

Effects of nutrients application on microbial biomass C formation

Effects of nutrients application on microbial biomass C in regosol are presented in Table 2 and Fig. 1. The

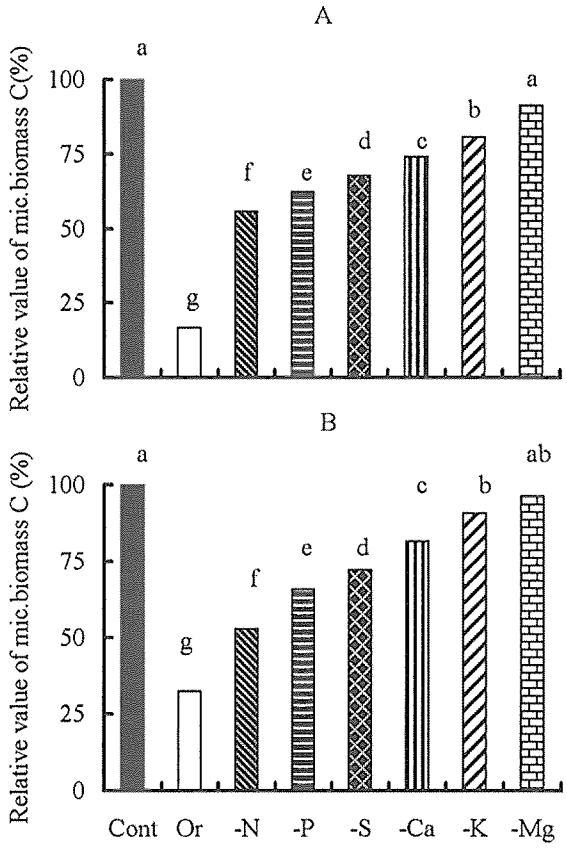

Figure 1 Relative value of microbial biomass C at 5 days (A) and 10 days (B) after nutrients application. Treatment with the same letter are not significantly different ($P<0.05$)

amount of microbial biomass C of each plot markedly increased by each nutrient application and were maximum at 5 days after treatment. The amount of microbial biomass C were significantly different among mineral nutrients. That was the highest at the control, higher at −K and −Mg plot and lower at −Ca, −S and −P plot, being the lowest in −N plot (Table 2).

At 5 days after nutrient application (5 d incubation), the amount of microbial biomass C in the control was the highest (414 mg C kg^{-1} soil) among the treatments. In comparison with the control, relative values of microbial biomass C of each plot was the lowest at −N plot (55%), followed by −P (62%), −S (67%), −Ca (74%), −K (80%) and −Mg (91%) plot, respectively (Fig. 1A).

At 10 days after nutrient application (10 d incubation), the amount of microbial biomass C in the control was smaller (142 mg C kg^{-1} soil) than that of 5 d incubation. This might be mainly due to the reduction of C supply for microbial biomass. However, relative value of microbial biomass C of each plot to the control was quite similar to those of 5 d incubation. That was the lowest at −N plot (52%), followed by −P (65%), −S (72%), −Ca

(81%), −K (91%) and −Mg (96%) plot, respectively (Fig. 1B). These results suggest that not only C and N but also P, S and Ca application is essentially required for microbial biomass formation in the regosol of Japan. While, there were a little effects of K and Mg application on microbial biomass formation.

In the case of plant production on regosols, N, P and Ca fertilization is generally required because of poor fertility of regosol. These might be due to the balance between nutrients requirement of plant growth and/or microbial biomass formation and nutrients supplying capacity of soils. Mineral (N, P, K and Ca) concentration in the microbial biomass was estimated about 70, 60, 50 and 7 $\mu g\ g^{-1}$ mic (Diaz-Ravina, et al. 1993). These values were higher than those of plant tissue, especially in P. Marumoto (1984) and Insam et al.

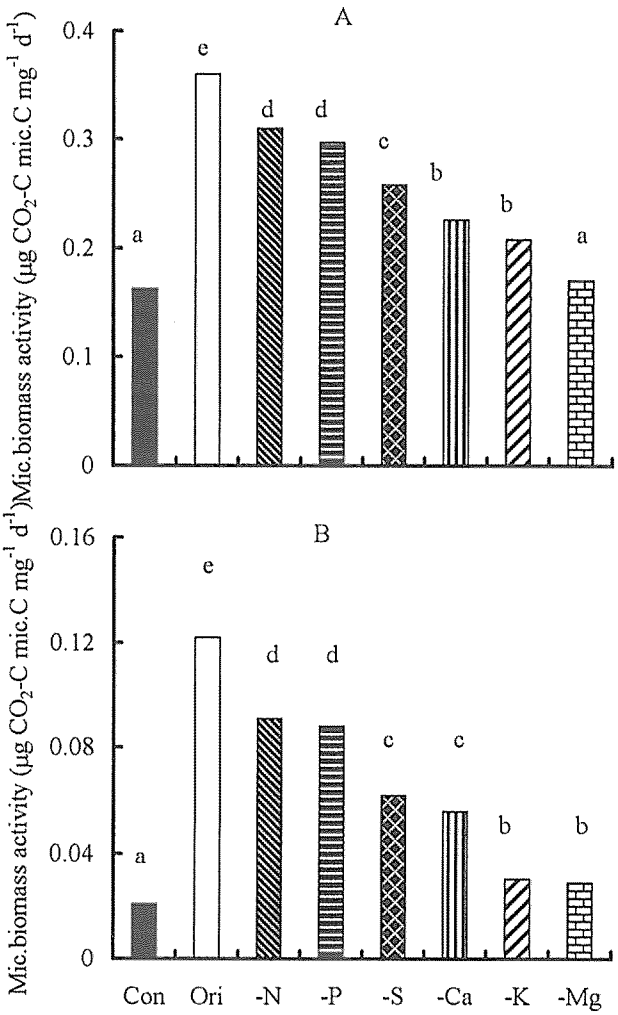

Figure 2 Microbial biomass activity at 5 days (A) and 10 days (B) after nutrients application. Treatment with the same letter are not significantly different ($P<0.05$)

(1991) reported that the microbial biomass formation was significantly correlated with soil organic C. The largest significant difference in the −N plot compared with the control suggested that the N application to the soil could not be avoided in the formation of microbial biomass. Azam et al. (1988) reported that immobilization of inorganic N is faster in the presence of an energy source, especially if the source (e. g. glucose) is readily available. Sparling et al. (1985) indicated that nutritional variations of extractable soil phosphorus and microbial carbon were significantly positively correlated.

However, there are little information about quantitative balance between nutrients requirement for microbial biomass formation and nutrients supplying capacity of soils and little information concerning other nutrients, such as S, K, Ca and Mg. Furthermore, up to now, the nutrients concentration in soils to be required for microbial biomass formation has not been understood well.

Effects of nutrients application on microbial biomass activity

Effects of nutrients application on microbial biomass activity are presented in Fig. 2. The specific respiration of microbial biomass (called metabolic quotient of CO_2 by Anderson and Domsch, 1985) is calculated as the ratio of CO_2–C evolved during incubation per mg microbial biomass per day. The microbial activity of all treatments were higher at the 5 d incubation (Fig. 2A) than those of 10 d incubation (Fig. 2B) and were significantly different among the treatments. Among the treatments, the microbial activity was the lowest in the control, low in −K and −Mg plot, high in −S , −Ca, −N and −P plot, and the highest in the original plot. These order was quite similar at both sampling day (5 d incubation and 10 d incubation). These results suggest that a larger amount of microbial biomass is becoming inactive, while a lower amount of microbial biomass is remaining in an active state. Santruckova and Straskraba (1991) found that the specific respiration decreased when the amount of microbial biomass increased. It seems that the microbial activity decreased with increasing of microbial biomass C. This reason is not clear, but the decreases of microbial biomass activity might be caused by limitation of nutrient or by the inhibition of high concentration of CO_2 produced by the microbial biomass population.

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土壌微生物バイオマス形成に及ぼす無機養分の影響

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貧栄養土壌のマサ土における土壌微生物バイオマスの形成に必要な無機養分を比較検討するために，土壌培養実験を行った。処理は，N, P, K, S, Ca, Mg 全てを添加した完全区と完全区から各要素を除いた −N, −P, −K, −S, −Ca, −Mg 区の7処理区を設け，マサ土を最大容水量の60%に水分調整した後，各処理区とも無機養分と 1 Kg 土壌当たり 1000 mg C をグルコースで同時に添加し，25℃で10日間培養し，5日目と10日目の微生物バイオマス量と土壌呼吸量を測定した。

微生物バイオマスは，養分添加によって5日目まで増加しその後低下したが，5日目，10日目のいずれにおいても，同様な処理間差異が認められた。すなわち微生物バイオマス量は，完全区で最も高く，Mg, K, Ca, S, P, N 欠如区の順に低下し，各処理区の完全区に対する相対バイオマス量は，Mg, K, Ca, S, P, N 欠如区で各々91–97，80–91，74–81，67–72，62–66，52–55%と N, P, S, Ca 欠如区で著しく低かった。また微生物バイオマス活性はバイオマス量の多い区ほど低い傾向が認められた。

これらの結果から，マサ土における土壌微生物バイオマスの形成には，C や N ばかりでなく，P, S, Ca などの供給が必要であることが明らかとなった。

キーワード：土壌微生物バイオマス，マサ土，無機養分，呼吸活性