RESEARCH ARTICLE

Microbial biomass C and N dynamics, and ^{15}N incorporation into microbial biomass under faba bean, canola, barley, and summer fallow in a Gray Luvisol

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Abstract: Microbial biomass C and N dynamics was studied of the soils cropped to faba bean, canola, barley and summer fallow in the Breton Plots in Canada. Stable isotope ^{15}N was used to trace the incorporation of ^{15}N into microbial biomass fraction. Flush N was calculated in three ways to compare discrepancies that may exist in results widely published. Crop and cropping had significant effects on microbial biomass C and respiration C of treated soils. Microbial biomass C estimated in faba bean plots was higher than that in canola, barley or summer fallow by 44, 39 and 167% on the average of four samplings. A peak was observed for canola, barley and summer fallow around July 24, but it was not evident for faba bean. Flush N was not significant upon treatments, and ^{15}N incorporated into the flush N fraction and ^{15}N excess varied with treatments. Among three approaches of calculating flush N, we found that NH_4^+ in fumigated less the nonfumigated soils yielded the best results, NH_4^+ in fumigated without control resulting in deviation from considering a control by 1-2% in faba bean, 1-6% in barley and 1% in canola and summer fallow.
Keywords: microbial biomass; N dynamics; cropping systems; faba bean; Gray Luvisol; symbiotic nitrogen fixation

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1. Introduction

Soil microbial biomass, a biologically active fraction of the soil organic matter and being the driving force for nutrient cycling, governs the rate of turnover and mineralization-immobilization of organic substrates in soil (Cleveland et al., 1999; Jansson, 1958; Paul and Juma, 1981; Paul and van Veen, 1978; Pereira e Silva et al., 2011). Its role in the transformation of organic matter is a crucial one despite its small quantities of biomass, only 1-2% total organic C and 3-5% total N of those in soil. Microbial biomass in soil has been studied intensively with ^{14}C and ^{15}N tracer techniques (Paul and Juma, 1981; Paul and van Veen, 1978), kinetics and mathematical models were developed to simulate microbial biomass on mineralization and immobilization of nitrogen in culture conditions (Van de Werf and Verstraete, 1987a, b, c), cropped field (Paul and Juma, 1981) and grassland system (McGill et al., 1981) based on experimental data in some degrees. Models have provided insight of control mechanism during biochemical processes, which are hardly to quantify due to methodological problems (van Veen et al., 1981). Soil available nitrogen is always being in a dynamic state and it is the microorganisms that play as a transformation agent and a source and sink for various nutrients in the transformating of nitrogen from the pool of soil organic matter to the available N in mineral forms, NH_4^+ and NO_3^- or incorporation of mineral N into soil organic N forms (Paul and Voroney, 1984). Microbial biomass regulates the transformation of soil organic matter and nutrient availability, both mineralization and immobilization processes are of concern in agricultural productions. The biological turnover through mineralization and immobilization between mineral and organic forms of nitrogen was regarded as an internal cycle of nitrogen in soil (Paul and Juma, 1981; Stevenson, 1986). However, activities of soil microbial biomass are always limited by the availability of C source in agricultural soil (Lynch and Panting, 1980a, b; McGill et al., 1986) and the environmental factors may place a restraint on its activity. Therefore, the two opposite processes of mineralization and immobilization are independent on the energy availability to microorganisms (McGill et al., 1975). Supply of decomposable organic matter is an indispensable prerequisite for these processes in the internal N cycle, but energy supply is not the only one because no N is available in N-free energetic materials to growing microorganisms. In so doing, microbes live on the inorganic N pool, they become suffering

from N deficiency and their activities will be limited when inorganic N pool is depleted.

Soil microorganisms have long been considered as a labile pool of C, N, P and S and consequently the turnover rates of these elements through the activities of microorganisms are very important for nutrient flow (Jenkinson and Ladd, 1981; Koponen et al., 2003). Hence, relationship between microbial biomass in soil and effectiveness of management on agricultural production will aid in further understanding and possibly manipulation of nutrient flow on a terrestrial scale (McGill and Myers, 1987; Lincoln and Vitousek, 2016). Microbial biomass in agricultural soil is affectted by crop rotation (McGill et al., 1986), tillage (Carter and Rennie, 1982; 1984; Lynch and Panting, 1980a, b), soil texture (van Veen et al., 1987) and alternation of moist and dry conditions (Bottner, 1985). Generally, exogenous substrates are in very low concentrations so that growth and activity of the biomass is restricted (Tateno, 1988), and the small amounts of substrate available are used for maintenance. Mechanisms and rates of turnover of nutrients through the soil biomass are still not well understood despite the need for an acceptable quantitative description of these processes to form the basis of improved nutrient management in different soils.

The objectives of this study were to estimate microbial biomass C and flush N dynamics of soils cropped to faba bean, canola, barley and a noncropped summer fallow; and to trace ^{15}N incorporation into microbial biomass fraction with ^{15}N tracer under cropping with three crops (faba bean, canola and barley) and noncropping (summer fallow) conditions. Results presented here are only part of an integrated study of C and N allocation among roots in three depths and above ground components of faba bean, canola and summer fallow. These detailed data will lead to further modeling of C and N distribution in the agronomic system and hopefully make a better understanding of the agricultural production system we are manipulating.

2. Materials and Methods

2.1. Soil Samples Used

This study was conducted in the Soil Science Plots at the Breton, which is 110 km southwest of Edmonton, Canada. Dark Gray Luvisols together with Gray Luvisols are predominant soils in the study area (Lindsay et al., 1968). The aspect is southwest. Experiment design was a four-treatment (faba bean, canola, barley and summer fallow) by four-replicate one and open-ended steel cylinders (30 cm in height and 20 cm in diameter) were used in this study. Four cylinders were placed in each replicate plot and the cylinder was specially designed with two 0.5 cm diameter holes on the opposite sides of the wall 1 cm below the upper end so that the holes were on the ground level after cylinders being pushed into the soil and could be functioning to discharge flooded water otherwise when there were a heavy rain fall. Further descriptions of experiment set up are available (Gu and McGill, 1988a, b).

Isotope N-15 in the form of urea was pulse-labeled four times on June 19, July 8, July 24 and August 19 in 1987 to surface soil at 2 cm depth within the cylinders using syringe with needle. Soil samples were taken 4 times during the growing season on July 8, July 24, August 19 and September 1, 1987 from the plots and analyses of soil microbial biomass C and N were conducted with composite samples of four replicates from each treatment for depth 0-10, 10-20 and 20-27 cm. These soil samples were kept at 4°C after sampling till analyses for microbial biomass C and N about four months later from the first sampling. A set of soil samples were taken for the whole plots to provide chemical properties analyses at the last sampling date (September 1, 1987), and chemical properties of the soil were presented in Table 1.

Table 1. Flush of N in the study plot of the whole growing season

	Jul. 8		Jul. 24		Aug. 19		Sep. 1	
	UF	FG	UF	FG	UF	FG	UF	FG
	(μg/g)							
Faba bean								
NH_4^+	1.03	21.59	0.61	21.8	0.94	22.1	0.97	25.52
NO_3^-	12.12	9.06	8.31	5.26	6.53	4.3	12.4	6.5
Canola								
NH_4^+	1.01	23.54	1.5	20.69	0.7	23.1	0.74	26.1
NO_3^-	6.33	4.87	7.1	3.89	6.44	5.23	8.21	5.34
Barley								
NH_4^+	0.27	25.01	0.46	20.75	0.74	25.4	1.1	24.89
NO_3^-	6.49	4.17	8.1	5.65	4.47	4.38	9.49	7.71
Fallow								
NH_4^+	0.59	22.24	0.49	20.03	0.67	21.8	0.55	21.81
NO_3^-	19.8	15.11	14.79	13.88	6.46	6.16	7.64	8.86

On each sampling, cylinders with soil inside were taken out of the plots and subsampling was conducted in laboratory after transported back (Gu and McGill, 1988b). One quarter of the total soil inside the cylinder was dissected into three depths (0-10, 10-20 and 20-27 cm) for later microbial biomass assay. These soil samples were passed through 10-mesh sieve and visible root were taken out. Soils were lefted over night at 22°C before carrying out fumigation assay. Chloroform fumigation incubation technique (CFIT) was used in this study to measure the proportion of total C and N mineralized from the microbial biomass (Jenkinson and Powlson, 1976a). Soils were extracted with 2 M KCl on the same day when fumigation was conducted and also the day after a 10-day period of incubation at 22°C for fumigated and non-fumigated soils.

2.2. Biomass C and N Determination

Twenty-five gram of soil in equivalent to dry weight was taken from each treatment 0-10 cm soil samples, which was a composite of the four replicates in plots at each sampling date. Two samples were fumigated and another two were left as controls. CO_2 evolved was trapped in 20 ml of 0.25 M NaOH in 2 L Karr jar and titrated with 0.5 M of HCl on a Memotitrator (Mettler DL40RC, Switzerland) after adding 5 ml of 15% $CaCl_2$ to precipitate trapped CO_2 as $CaCO_3$ precipitate. The quantity of microbial biomass C is calculated by dividing the difference in CO_2-C evolved during a

$$\text{Biomass} - \text{C} = \frac{\text{CO}_2 - \text{C in fumigated soil} - \text{CO}_2 - \text{C in nonfumigated soil}}{0.411} \quad (1)$$

10-day incubation of fumigated and non-fumigated soils by a factor 0.411 as Equation 1 (Anderson and Domsch, 1978).

Mineral N in the extract, which was extracted with 2 M of KCl for soils before fumigation, and fumigated and non-fumigated soils after fumigation and incubation, was determined with steam-distillation with MgO (0.5 g) for $NH4^{-}_{+}N$ and followed with Devarda's alloy (0.2 g) for (NO_3^- +NO_2^-)-N (Bremner, 1982) after cooling down near room temperature. Biomass N was not calculated due to the discrepancies in reported data (Voroney and Paul, 1984); instead, flush N was used throughout in this paper. Flush of N was calculated in three ways and they are:

$$\text{Fn} = \text{F}(\text{NH}_4^+ + \text{NO}_3^-)_{10} - \text{F}(\text{NH}_4^+ + \text{NO}_3^-)_0 \quad (2)$$

$$\text{Fn} = \text{F}(\text{NH}_4^+)_{10} - \text{F}(\text{NH}_4^+)_0 \quad (3)$$

$$\text{Fn} = \text{F}(\text{NH}_4^+)_{10} \quad (4)$$

No attempt was made to convert flush of N to biomass N due to different factors applied to published data. McGill et al. (1981) reported variation of N content could be 2- to 4-folds between bacteria and fungi. NH_4^+ from both steps of distillation was trapped in 5 ml of 2% boric acid and then titrated with dilute H_2SO_4 on a Memotitrator (Mettler DL40RC, Switzerland). All samples were acidified with 2 drop 0.5 M of H_2SO_4 after titration to a pH approximately 3.4, and dried on an 800°C sandbath. Salts were re-dissolved and transferred to culture tubes and oven-dried at 65°C for N-15 analysis on the ANA-SIRA N-15 Mass Spectrometer (Nitrogen/Carbon/Sulphur analyzer, VG ISOGAS, England). For those samples containing less than 100 µg N, a modified diffusion method was applied in this study (procedures are described in the following section). Samples were run in duplicate. During the steam distillation, the recoveries were 98% and 93% for ammonia and nitrate respectively in a step-wise sequence as for samples; whereas total mineral N was determined with the same standard solution, and a recovery of 97% was obtained. This is agreed with distillation ammonia and nitrate separately or at once proved that method is reliable. Flush of mineral N was calculated and presented. No attempt was made to convert flush of N to biomass N due to different factors applied to published data.

2.3. N-15 Diffusion Technique

For samples that contained less than 100 µg N/sample during acid titration, N-15 diffusion technique was applied (P.D. Brooks, personal communication), this is a modified procedure from Turner and Bergersen (1980). A solution (20-30 ml) was placed in a 140 ml plastic specimen container, and a 6 mm (Diameter) disk , cut from Whatman GF/D glass fiber filter paper with a paper punch, was put on a #22 solid PVC coated wire (type MW-U, MIL-W-76B) in the middle portion of this wire. Ten µl of 2.5 M $KHSO_4$ were pipetted onto the disk and a small scoop MgO (0.2 g) was added to solution in the container, then put the wire with disk onto the plastic container with both sides of the wire extended onto the wall of the container, so that the disk was suspended above the solution. Cap the container tightly and swirl the container carefully to let MgO becomes well mixed with solution. The containers were left at room temperature (22°C) for 6 days for diffusion of NH_3 onto the $KHSO_4$ moist disk. The MgO raises solution pH so that the NH_4^+ becomes NH_3, which is then volatilized. The NH_3 gas was absorbed by the acid $KHSO_4$ on the filter paper and converted back to NH_4^+ before uncap the container and transfer wire with disk on it to a stereoform for stands. If paper disk absorbed too much moisture during the 6-day diffusion and water droplet is visible on the disk, it is suggested that carefully tilt the holding wire so that lightly strike with thumb to the lower end of wire to let disk moved to near the lower end of the wire, then bend the wire from the middle part to 90-120°. The wire can be pushed into stereoform by twister while disk is staying on the horizontal portion of the wire. As soon as all wires with disks were put on the stereoform, stereoform was transfered to a desicator with a 500 ml beaker containing 400 ml concentrated H_2SO_4 in it and sealed. Four days later, N-15 analyses were carried out on these disks using the ANA-SIRA N-15 Mass Spectrometer (Nitrogen /Carbon /Sulphur analyzer, VG ISOGAS, England).

2.4 Statistical Analysis

Statistical analysis was conducted for ANOVA (P3V), multiregression (P2R) and principal component analyses (P2R) on NH_4^+, NO_3^- in soils before fumigation and soils fumigated and nonfumigated after 10 days incubation using BMDP statistical software (Dixon, 1983). Three crops together with summer fallow were categorized as treatment whereas four sampling dates were considered as another treatment. Multiple comparison of Student-Newman-Keuls was carried out when a significant difference was found upon treatment effect of ANOVA.

3. Results

3.1. Soil Microbial Biomass C and Respiration

Microbial biomass C in soils was calculated from difference between CO_2-evolved of fumigated less non-fumigated soils divided by a factor 0.411 (Anderson and Domsch, 1978) during a 10-day incubation as described in Equation 1. Results varied with the progress of growing season and cropping system, a general pattern was a bell-shaped curve on the four samplings for the four treatments (Figure 1). Analysis of variance showed that treatment and sampling date effects were significant on microbial biomass C ($p<0.01$). Interactions of treatment and date were also significant on microbial biomass C ($p<0.1$). Microbial biomass C in cropped soils was higher compared with summer fallow one except

for the last sampling date, ranged between 131.04 μg C/g soil and 429.08 μg C/g soil in summer fallow, and between 272.1 μg C/g soil in barley on August 19 and 836.34 μg C/g soil in canola plots of Jul. 24 (Figure 1). On the last sampling, microbial biomass C was significantly higher in faba bean plot than that in canola, barley or summer fallow plots; whereas there was no significant difference among the latter three. At that date, microbial biomass C in faba bean plot was 135, 84 and 170% higher than that in canola, barley and summer fallow, respectively.

Microbial biomass C of faba bean plot did not vary significantly throughout the four samplings in the growing season between 574.99 to 674.02 μg C/g soil while that of barley plot almost remained the same during the first two sampling dates then decrease significantly on the third sampling date and increased significantly on the last sampling. In contrast, biomass C of canola plot was increased significantly by more than two times after the first sampling and then decreased by more than two times after the second sampling date to the level similar to first sampling. The pattern in summer fallow plot is much similar to that in canola's; at each sampling date, fallow plot had significantly less biomass C among treatments, except for the last sampling. On Jul. 8, biomass C in faba bean and barley plots was significantly higher than that in canola and summer fallow plots. Compared with summer fallow plot, microbial biomass C was 2.6, 1.2 and 2.8 times higher than that in faba bean, canola and summer fallow on the average of four samplings. About two weeks later, the biomass C in cropped plot was reversed and biomass C in canola plot was significantly higher than in the other two cropped soils, microbial biomass C of canola plot was 2 times as high as that in fallow and 24% higher than that in faba bean and 39% higher than in barley plots. On Aug. 19, biomass C in canola and barley plots dropped to about one half that in faba bean plot and biomass C of the last sampling remained almost the same trend as third sampling except for the significantly increase in barley and fallow plots.

The CO_2-C evolved during a 10-day incubation of the non-fumigated soils was taken as soil respiration C. Sampling date and treatment effects were significant for $p<0.01$ and $p<0.1$, respectively. Their interaction was not significant ($p<0.1$). Over the four samplings in growing season, respiration C from faba bean plots was lower than that from the canola, barley or summer fallow plots except for the second sampling and there was no significant difference among treatments on that date. In the growing season, respiration C in faba bean plots was significantly decreased from 131.22 μg C/g soil of the first sampling to 29.30 μg C/g soil of the third sampling. The respiration C of canola plot had a pattern that decrease on the second sampling and then maintained through Aug. 19 and further increase on Sep. 1. Respiration C of canola plot was decreased by 77%, 79% and 36% on the second, third and forth samplings compared with the first sampling. In contrast, the decrease was 51%, 6% and 28% in barley plot and 68%, 51% and 37% in summer fallow. All four treatments had pattern that decreased

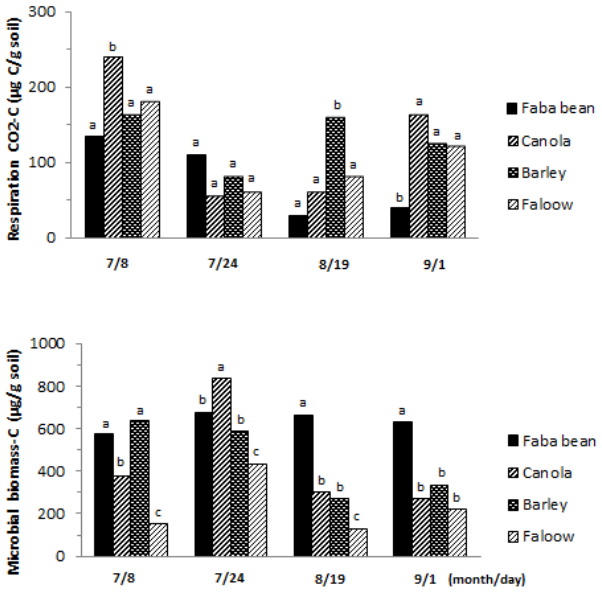

Figure 1. Microbial Biomass C and respiration C in 0-10 cm depth over the growing season. a-c means followed by the same letters do not differ significantly on one date ($p<0.05$)

on the second sampling (Jul. 24) and then increasing again as observed on Aug. 19 and extended increases to Sep. 1. On Jul. 8, respiration C of canola plot is significantly higher than in faba bean, barley and fallow Plots (Figure 1), but no significantly difference in respiration C was observed on Jul. 24 for the four cropping system. On Aug. 19, respiration C from barley plot is significantly higher than the other three, while that from faba bean plot significantly lower than the others on Sep. 1.

Biomass C and respiration C showed decreasing in 10-20 cm depth compared with the quantity in 0-10 cm except for biomass C in fallow plot on the last sampling and respiration C in faba bean plot on the last sampling (Figure 2). The magnitude of biomass C decrease is more enhanced for faba bean followed by canola and barley. Compared with 0-10 cm depth, microbial biomass C decreased was by 58, 32 and 17% in 10-20 cm depth soils cropped to faba bean, canola and barley; but increased by 4% in summer fallow plot. There was a slightly increase in microbial biomass C in the fallow plot. The respiration C decreased quite dramatically in 10-20 cm depth for canola, barley and summer fallow, but increased in faba bean plot. The decreases were 50, 40 and 41% in 10-20 cm depth soil cropped to canola, barley, and summer fallow in comparing 0-10 cm, but increased by 91% in 10-20 cm of faba bean compared with 0-10 cm.

3.2. Flush of N Released

Sampling effects on the flush N was significant ($p<0.1$), but treatment, and interactions of treatment and date effects were not ($p<0.1$). Variation of flush N was between 16.96 - 24.54 μg N/g soil for the four samplings of four treat-

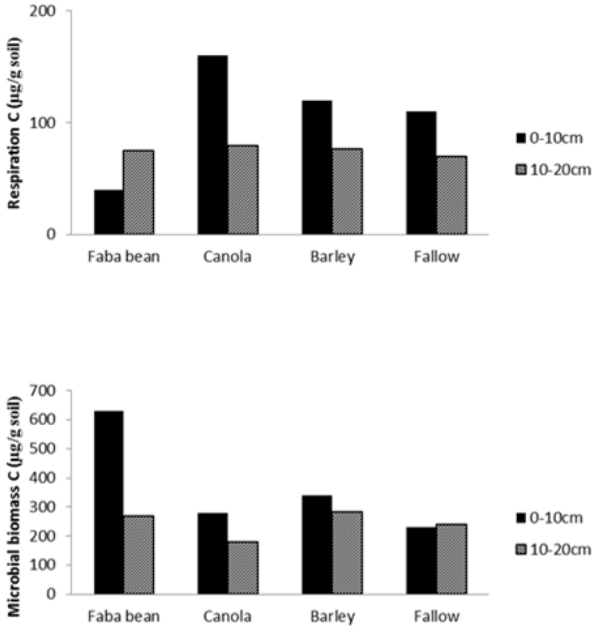

Figure 2. Microbial biomass C and respiration C for 0-10, 10-20cm depth on the last sampling

ments. The average values of four samplings were 18.30, 20.48, 20.70 and 19.73 µg N/g soil for faba bean, canola, barley and summer fallow, respectively. Flush N was calculated in three ways: first, taking difference of NH_4^+ and NO_3^- between fumigated and non-fumigated soils at day 10; second use difference of NH_4^+ in fumigated and non-gumigated soils at day 10; and third use NH_4^+ in fumigated soil at day 10 without subtracting a non-fumigated control. Data shown that the latter two without considering NO_3^- correlated very well and range varied 1% for faba bean, 0% for canola, 6% for barley and 0% for summer fallow. Results obtained without control were higher than the ones control was considered. On the other hand, variation was 23% for faba bean, 20% for canola, 23% for barley and 40% for summer fallow for the ones both NO_3^- and NH_4^+ were taken into account foe estimation of flush N. These results were lower than proposed calculating method in Equation 3. Second calculation was used in the data reported in Table 2. Quantitative N-15 data showed there was a corresponding increase of N-15 incorporated into microbial biomass fraction except on July 24 of canola plots. The increase of N-15 incorporation over time for faba bean was 71, 41 and 91% between samplings; it was 14, 32 and 146% for canola; 61, 74 and 16% for barley; and 11, 85 and 26% for summer fallow (Table 2). Microbial biomass C was significantly lower in the summer fallow plots, incorporation of N-15 into microbial biomass shown similar trend.

Incorporated ^{15}N in flush N increased after each addition of ^{15}N except for one case, on the second sampling in canola plot. Incorporated ^{15}N ranged between 48.63-224.76, 57.29-185.80, 45.76-149.13 and 44.07-113.35 ng

Table 2. N-15 of Mineral-N fractions in the study plot of the whole growing season

	Jul. 8	Jul. 24	Aug. 19	Sep. 1
		Faba bean		
NH_4^+	0.44457	0.433945	0.6334	0.38555
NO_3^-	1.149085	1.43643	1.218645	0.50247
		Canola		
NH_4^+	0.44184	0.428905	0.486455	0.382595
NO_3^-	1.08707	0.771315	0.81752	0.497785
		Barley		
NH_4^+	0.42024	0.435665	0.512215	0.377305
NO_3^-	0.727845	0.91705	0.97652	0.45992
		Fallow		
NH_4^+	0.43748	0.439443	0.51869	0.381095
NO_3^-	1.955475	0.51869	1.11539	0.48243

^{15}N/g soil for faba bean, canola, barley and summer fallow plots. Quantities of ^{15}N in summer fallow were lower than that in cropped soils. On the first sampling, ^{15}N in microbial biomass fraction of summer fallow was 10, 51 and 4% lower than that in faba bean, canola and barley plots. It was 98, 64 and 32% on the last sampling. Flush N calculated with NH_4^+ in fumigated only was close to the one subtracting NH_4^+ in the control during 10 days of incubation, but yeilded slightly higher values for all treatments on the four sampling dates. It was found that the overestimation was higher by 1-2, 1, 1-6 and 1% in faba bean, canola, barley and summer fallow. Calculation with NH_4^+ and NO_3^- at 10 days for fumigated one less the nonfumigated one resulted in lower rather than those from NH_4^+ without considering NO_3^-. It can be lowered by 17-67, 7-27, 0-23 and 1-31% for faba bean, canola, barley and summer fallow plots.

^{15}N excess in microbial biomass N fraction was calculated with the three means using ^{15}N abundance in the atmosphere as a reference (Junk and Svec, 1958). ^{15}N excess increased over time for the four treatments except for canola on July 24. It was 66, 42 and 41% in faba bean; -4, 19 and 117% in canola; 96, 44 and 20% in barley and 22, 72 and 25% in summer fallow. The exception was also observed for quantity ^{15}N on that date. Estimated ^{15}N excess slightly lowered when NH_4^+ in fumigated was taken solely to calculate the biomass N than subtracting the NH_4^+ in the nonfumigated control in 10 days incubationm (Table 3). Percentage of underestimation was 2-3, 2-3, 1-2 and 1-2% for faba bean, canola, barley and summer fallow, respectively. ^{15}N excess was lowest when both NH_4^+ and NO_3^- of fumigated and nonfumigated ones were used in calculation. In this case, the underestimation was 7-39, 2-15, 0-10 and 3-28% for faba bean, canola, barley and summer fallow.

Significances were observed on NH_4^+ for date ($p<0.05$), fumigation ($p<0.01$) and their interaction effects ($p<0.05$), whereas treatment, interactions of treatment × date, fumigation × treatment, treatment × fumigation, and fumigation × treatment × date were not significant ($p<0.1$). Accumulation of NH_4^+ and slightly depletion of NO_3^- were evident

Table 3. N-15 in flush of mineral-N fractions in the study plot of the whole growing season

	Jul. 8		Jul. 24		Aug. 19		Sep. 1	
	UF	FG	UF	FG	UF	FG	UF	FG
Faba bean								
NH_4^+	0.47071	0.59648	0.553563	0.75257	0.56999	0.906775	0.708875	1.25962
NO_3^-	1.1147	1.151285	1.41304	1.313535	1.1458	1.09125	1.768195	1.67284
Canola								
NH_4^+	0.443045	0.652665	0.464005	0.645545	0.457825	0.69615	0.682315	1.08716
NO_3^-	1.01371	1.08869	0.83424	0.79708	0.80036	0.80036	1.371655	1.339125
Barley								
NH_4^+	0.432715	0.55037	0.459785	0.72286	0.876675	0.876675	1.08865	1.000285
NO_3^-	0.696825	0.707535	0.94775	0.93232	0.97076	0.97076	1.51801	1.53452
Fallow								
NH_4^+	0.460935	0.56712	0.43983	0.611265	0.507265	0.78511	0.53033	0.89018
NO_3^-	1.93631	1.951365	2.02473	2.102725	1.04454	1.09978	2.16149	2.30961

on cropped and non-cropped soils after fumigation (data not shown), increase in NH_4^+ caused by chloroform fumigation was approximately 21-93 folds. Largest amount of NH_4^+ released was found at the last sampling data for soils subjected to crop growing. Isotope N-15 date showed N-15 abundance in NH_4^+ was slightly enriched for non-fumigated soil after 10-day incubation comparing with N-15 before incubation. Fumigation effects had increased N-15 enrichment in NH_4^+ dramatically. CFIT does cause a release of biologically meaningful, immediate releasable organic N flush. For NO_3^- in soil, crop, date, and interaction between crop and date, fumigation effects were significant (p<0.01). Interactions of fumigation × treatment was significant (p<0.1), but interactions of date × fumigation, and fumigation × crop × date were not significant (p<0.1). Tendency was evident that N was mineralized and further nitrified to NO_3^- form for non-fumigated soil during a 10-day incubation, while NO_3^- level was lower in fumigated soils after a 10-day incubation (Table S1). N-15 enrichment of NO_3^- form was 2- to 3-fold that of NH_4^+ form for soil before CFIT (Table S2). Amount of NO_3^- accumulation was increased for non-fumigated soil after incubation, but the N-15 enrichment had a tendency to decrease. This indicates a non-labeled N source was immediate released or nitrified to NO_3^-. However, N-15 enrichment in nitrate was increased for fumigated soil after incubation although the total quantity had decreased. Therefore, a N-15 labeled N source had contributed to this increase.

Quantities of ^{15}N in microbial biomass fraction decreased in 10-20 cm depth comparing with that in 0-10 cm for all of the four treatments. The magnitudes of decrease was 10, 19, 32 and 14 times in faba bean, canola, barley and summer fallow, respectively (Table S3). Excess ^{15}N had similar trend as that for quantities of N-15, being decreasing in 10-20 cm. In contrast to quantities ^{15}N, excess ^{15}N changed in a narrower range and the magnitude was 6, 10, 15 and 8 times in faba bean, canola, barley and summer fallow(Table S4).

3.3 Extractability Ratio

Flush N was significant only for the sampling effects. Consequently, it is obvious that the source of NH_4^+ and NO_3^- is critical to elucidate the flush of N. Extractability Ratio (ER) was one way to characterize the sources of N (Juma and Paul, 1984; Legg et al., 1971).

$$\mathrm{ER} = \frac{\frac{\text{labeled N extarcted}}{\text{total N extracted}}}{\frac{\text{labeled total N}}{\text{total N}}} \tag{5}$$

It can be simplified to

$$\mathrm{ER} = \frac{\text{atm\% } ^{15}\text{N of extracted N}}{\text{atm\% } ^{15}\text{N of total N}} \tag{6}$$

Lower limits of it is zero and could be obtained of no labeled N was extracted. An ER $>$ 1 implies that N compounds extracted are relatively enriched in ^{15}N compared with enrichment of total N in soil. Generally, the ER of NH_4^+ form N was higher than that of NO_3^- when soil is tested without incubation. There was a tendency of decrease for NH_4^+ form N during the growing season, but not for NO_3^- form N. Compared with N-15 enrichments in NH_4^+ and NO_3^- after 10-day incubation for non-fumigated soil, ER was lower in NH_4^+ fraction while the ER was inceased in NO_3^- fraction. Highly labeled N was mineralized during this incubation period. The depletion of N-15 enrichment of NH_4^+ fraction in non-fumigated soil may imply that labeled fraction of NH_4^+ was undergoing nitrification process to NO_3^-. CFIT causes a rapidly flush of N in the form of NH_4^+, this was confirmed with ER data, the consistent increase of ER agreed with our pulse-labeling technique used in this study, whereas N-15 in NH_4^+ fraction before fumigation did not present this trend. In other words, CFIT gives a better understanding of immobilized N and origin of the released N form in soil.

ER in flush N calculated with Equation 3, in which the flush was the NH_4^+ in fumigated and non-fumigated soils after 10 days of incubation increased as growing season proceeded. The exception was found in canola plot and ER decreased by 6 and 2% on the second and third samplings (Table S5). ER was generally higher in faba bean than that in the other three and lower in the summer fallow. Range of ER was between 1.52-2.86 in faba bean; 1.59-2.45 in canola; 1.40-2.25 in barley; and 1.41-2.06 in summer fallow. The percentage increase between adjacent sampling times was 19, 17 and 35% in faba bean; -6, 5 and 47% in canola; 28, 16 and 8% in barley; and 4, 27 and 11% in summer fallow.

4. Discussion

4.1. Soil Microbial Biomass C and Respiration

Microbial biomass C estimated in faba bean plots was higher than that in canola, barley or summer fallow by 44, 39, and 167% on average of the four samplings, but canola and barley seemed to have similar microbial biomass. Legume, faba bean, supported a much higher microbial biomass than any of the other treatments, canola, barley or summer fallow. Barber and Martin (1976) had reported 18-25% of the total dry matter production of barley and wheat may be released by roots, which corresponded to 12-18% of the photosynthetically fixed C. Barber and Lynch (1977) observed that microorganisms on and around the roots could also enhanced the released of substrates. The cause may be both directly by the production of substrates which stimulate the process or indirectly by utilizing the exudates and preventing their built-up in solution, thus increasing outward diffusion. Martin (1975) used $^{14}CO_2$ feed wheat and found that 39% of ^{14}C translocated to the root was lost as root exudates, root lysates, mucilage, cell wall residues and intact plant cells. Summer fallow had the lowest biomass may be due to the shortage of energy supply for microbial development. Allocation of assimilated C from tops to roots could be as high as 50-85% for tallgrass (Dahlman and Kucera, 1968); similarly, 15-25% was found in roots and shoot bases and 17-25% was lost by underground respiration from wheat (Warembourg and Paul, 1973). Sauerbeck and Johnen (1977) had separated CO_2 output from soils to soil respiration, root respiration and root decomposition respectively. Their results indicated that rhizodeposition of organic matter during growth period of wheat exceeded the mechanical separated root by about 20%. It is not surprising that cropping does affect microbial population as reported by Lynch and Panting (1980a, b).

Root dry matter of faba bean was 3 times as much as that in canola or barley whereas no significant difference between canola and barley was observed (Gu and McGill, 1988a). In contrast, microbial biomass of faba baen plots was 39-44% higher than that in canola or barley plots. Major differences exist for total C reduced and thereafter the microbial population established between legume (faba bean) and nonlegumes (canola and barley). Reduced C source is a primary limiting factor for microflora developing in such oligotrophic environment as in soil (Taneno, 1988). Martin (1975) found that about 15% of the labeled material in the leachates behaved as neutral sugar, the remainder as charged complex. Monreal et al. (1981) had observed that water soluble C in Andept and Mollisol was comparatively lower than that needed for microbial maintenance and growth. The concept of maintenance energy was brought up again by Mallette (1963) and the theory was evaluated with *Escherichia coli* (Marr et al, 1963). In culture condition, maintenance coefficient of 0.07 g glucose/(g dry wt)/h had been obtained (Pirt, 1965). If assume an average of microbial biomass 300 μg C/g soil as in barley and canola plots, 10.7 mg C/g soil /h is needed for maintenance only. In contrast, water-soluble C in soil was only 20 μg/g soil (Dinwoodie, 1988). It should be kept in mind that maintenance energy of soil microorganisms was not quantified yet and it is only the general concept applied here for further consideration. Despite of the draining of root exudates, symbiotic N fixation in faba bean provided external N input into the plants and may enhance faba bean photosynthesis and developing, and consequently the flourishing of microbial population.

Microbial biomass C was accounted for 2.9, 2.0, 2.1 and 1.1% of soil organic carbon for faba bean, canola, barley and summer fallow and these values of cropped soils are consistent to the 2-3% reported from mineralization-immobilization study on a Black Chernozemic soil in Saskatchwan (Paul and Juma, 1981) and the results from the same long-term rotation plots for forage rotation effect in Alberta, Canada (McGill et al., 1986) except for the legume faba bean. Biederbeck et al. (1984) reported a 2.4% of soil organic carbon was contributed from microbial biomass C in a 12-year continuously wheat rotation receiving P only and 1.1% of the soil organic C in fallow-wheat rotation in Saskatchewan. Jenkinson and Powlson (1976a) showed that stubbled soil contained considerably more biomass C than arable soil and the biomass constitutes a higher proportion (3.7%) of the total soil organic C. Since the microbial community in soil environment changes in responding to crop species, partially ready available substrate. The relatively higher biomass C in faba bean plot is a direct indication of the results from microbes and crop interactions. Lynch and Panting (1980a) had observed that correlation exist between soil microbial biomass and root growth and rooting density of the crop. Foster and Rovira (1976) found that older roots of wheat at the flowering stage showed consistent development of microorganisms both in the rhizosphere and in the outer cortical cells and cell walls.

Respiration CO^2 evolved during 10 days of incubation of the non-fumigated soil can be an estimate of soil biological activity. Respiration C of canola and barley plots did not differ significantly between 128.83 - 132.92 μg C/g soil, but fallow plot had lower respiration C 110.89 μg C/g soil. These data were in good agreement with the results reported on barley (Klemedtsson et al, 1987). Surprisingly, respiration C from faba bean plots was even lower than that in summer fallow one whereas microbial biomass C of faba bean plots was the highest among all the treatments in general. This suggests that the large number of viable microbes presenting in cropping soils does not give information on metabolic activity or stage of these microorganisms. Nannipier et al.(1978) showed that using one or two indices to estimate microbial biomass activity is too simplistic and not sufficient for interpretating the microorganisms in the soils. Results of Dinwoodie (1988) had reported 43% of the microorganisms in the Gray Luvisol cropped to barley was active. Root exudates stimulates population increase but most of these organisms are either dormant or starved due to lack of available energy supply (Lynch and Panting, 1980a, b).

Microbial biomass C and respiration C decreased in all treatments as increasing in soil depth from 0-10 cm to 10-20 cm. Similar trend was obtained by Dahlman and Kucera

(1968) using ^{14}C radioactive tracer. Results indicated paralleled allocation of assimilated C to plant roots in various depths. The decrease in soil organic matter, available carbohydrate and limiting factor for microorganism development, limitation of root biomass in lower depth and exudation mainly from the basal regions of the root prevent microflora development in soil (Clark and Paul, 1970). Close relationship between energy supply and crop growing had already been reported (Christie et al., 1986; Powlson et al., 1987).

4.2. Flush N Released

Fumigation on release of NH_4^+ form of N is evident and 20-90 folds of NH_4^+ accumulation was observed from our field samples. The trend is in good agreement with initiative study by (Jenkinson and Powlson, 1976a) and more detailed study on 37 soils at 3 depths (Brookes et al., 1985). Results from Brookes et al. (1985) indicates the flush of N release was accumulated in the form of NH_4^+, but the range (10-100) of magnitude between non-fumigated and fumigated soils were one magtitude wider than ours (20-90) from four cropping treatments and four dates. Further calculation from our results showed range of NH_4^+ released on a magnitude between 9.7 and 16.5. Brookes et al. (1985) proposed this fraction of NH_4^+ is probably attributes from cytoplasmic component of the soil microbial biomass. When N mineralized from microbial metabolites, it remained as NH_4^+ (Jenkinson and Powlson, 1976b). However, a small decrease of NO_3^- was observed caused by chloroform fumigation, and confirmed that nitrifying bacteria were killed during the fumigation process and had not recovered because there was no increase in NO_3^- in soil after 10-day incubation. Jenkinson and Powlson (1976b) proposed the possible mechanism similar to that occuring in irradiation damaged soil, in which case there is also an increase of NH_4^+.

Treatment effects were not significant (p<0.1) though sampling effects were (p<0.1). Our results may be due to the sampling depth 10cm and the time for samples storage. Significant difference can be resulted only when shallow surface samples were compared and nitrogen immobilization during biomass determination should be considered in interpretation (Nannipieri, 1984). Storage of samples for 24 hours before measurement produced insignificant changes in the microbial biomass, but further storage of soil samplies on microbial biomass estimated is not available from the literature. Lynch and Panting (1980a) had observed that sieving soil reduce the estimated microbial biomass, thus method to measure soil microbial biomass is not absolute estimation. A further limitation is the uncertainty of fumigation efficiency and thereafter the K_c value. Chloroform fumigation technique was based on a series of assumptions (Jenkinson and Ladd, 1981), one of them is that mixed microorganisms killed decomposed to an equal extent in various soils. Furthermore, K_c and K_n values may be applied to qualitatively and quantitatively different biomasses. If this assumption is true, then a correlation between flush of carbon and flush of N during the 10-day incubation should be resulted. Our results has shown that generally very poor correlation between them was observed for barley, faba bean, and fallow, only in case of canola the correlationship reached 0.60. In soil environment, fungi appears earlier in quantity than bacteria and their compositions are distinct. The hetereogeneity of substrates (killed microbial biomass) for flurishing bacteria after fumigation makes quantitatively and qualitatively estimation of microbial biomass difficult (McGill et al., 1981). The C/N ratio is one of the governing factor in decomposition processes and cytoplasmic component is mineralized quickly except cell walls, which are often very highly carbonaceous. The C calculated from CO_2 evolved and N mineralized are the portion of microbial cell components that decomposed in a 10-day incubation. Therefore,"microbial biomass C" and "microbial biomass N" may not reflect the partially recalcitrant nature of microbial cellular structure, especially for fungal hyphae. Despite partitioning bacteria and fungal biomass is reasonable, lacking of reliable information about the details in regards to microbial biomass dynamics makes modelling dynamics of bacteria and fungal biomass quantitatively impossible.

Microbial biomass N can be calculated in several ways (Paul and Voroney, 1984), this includes using a control of 0-10 day or 10-20 days, and no control at all for the fumigated soils. Flush N, instead of microbial biomass N, was reported (McGill et al., 1986). Three calculations were conducted to estimate the flush N (Equation 2, 3 & 4) and results of quantities of ^{15}N incorporated into the microbial biomass of Equation 3 were presented (Table 2). In this calculation, flush N was taken as the NH_4^+ caused by chloroform fumigation less the amount of NH_4^+ in the nonfumigated control during 10 days incubation. Among the several means to calculate the microbial biomass N, NH_4^+ is the key cation to be analyzed and estimation from NH_4^+ gives consistent results even though control is eliminated. Generally, ^{15}N incorporation into the microbial biomass of summer fallow was lower than that in faba bean, canola or barley plots. This is in a good agreement with microbial biomass dynamics. On the last sampling, amount of ^{15}N in flush N was 224.76, 185.80, 149.13 and 113.35 ng ^{15}N/g soil. The ^{15}N excess in microbial biomass fraction is lower in summer fallow than that in cropped to faba bean, canola or barley. On the last sampling, ^{15}N excess was 0.91508, 0.73268, 0.62673 and 0.53326% in faba bean, canola, barley and summer fallow. Results suggested either a higher turnover rate was operating in cropped soil or ^{15}N labeled exudates were generated for microbial growth. Faba bean is a legume crop and N fixation can be as higher as 184-198 kg N/ha/yr (Gu and McGill, 1988b). ^{15}N labeled NH_4^+ or NO_3^- must be metabolized then distributed among various components of a crop, therefore the higher ^{15}N excess found in microbial biomass must be associated with interactions of crop root in soil and microorganisms. Unless highly ^{15}N enriched compounds were released to microorganisms in soil rhizosphere or the turnover rate is high then biomass N would not be so enriched in a legume. Gu and McGill (1988b) found that about 18-22% N in roots had an origin from atmosphere and 79% for N in tops, so N in rootd had high N-15 excess and so will the metabolites released or exudated. However, the single contribution could

not explain the higher ^{15}N excess. In addition, turnover rate must be considered to account for the high ^{15}N excess found in soil cropped to faba bean.

Quantitative data of ^{15}N incorporated into microbial biomass fraction in 10-20 cm shown that the decrease was as high as 14-32 times compared with 0-10cm. In 10-20 cm, ^{15}N excess was higher in faba bean plot, followed by canola and summer fallow, while barley had the lowest ^{15}N (Table 4). This may be due to the maturity of barley had been reached before the last sampling and canola was about fully mature, but faba bean was in grain-filling stage. ^{15}N was applied in surface soil four times, quantities and excess ^{15}N would be partially the results of ^{15}N movement down to the lower depth. Apart from this, physical root elongation and soil animal movement can also contribute to the enhanced ^{15}N movement in soil. Generally, ^{15}N movememt in non-cropped soil can be considered as the same rate. Results can be root exudation of ^{15}N enriched polymers into the rhizosphere soil and microorganism feed on these avaliable energy source; and also may be the physical action of roots and dynamics of is as growing left root channels thereafter accelerate mass transfer.

Table 4. Extraction ratios for mineral N at time zero, and N mineralized in 10-day incubation without CFIT and 10-day after CFIT

	Time=0		Non-fumigated, t=10d		Fumigated soil, d=10	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
			Jul. 8			
Faba bean	2.89	1.12	1.18	2.8	1.5	2.9
Canola	2.8	1.14	1.15	2.61	1.68	2.8
Barley	1.85	1.07	1.08	1.78	1.4	1.8
Fallow	4.85	1.08	1.15	4.8	1.41	4.84
			Jul. 24			
Faba bean	3.43	1.06	1.32	3.37	1.8	3.13
Canola	1.9	1.05	1.14	2.05	1.58	1.95
Barley	2.26	1.07	1.13	2.33	1.78	2.3
Fallow	4.61	1.04	1.05	4.82	1.45	5
			Aug. 19			
Faba bean	2.8	1.46	1.31	2.63	2.08	2.51
Canola	1.92	1.14	1.06	1.9	1.63	1.89
Barley	2.28	1.2	1.23	2.27	2.05	2.28
Fallow	2.64	1.23	1.2	2.53	1.86	2.6
			Sep. 1			
Faba bean	1.12	0.86	1.58	3.95	2.81	3.74
Canola	1.13	0.87	1.54	3.1	2.46	3.03
Barley	1.05	0.86	2.49	3.47	2.29	3.51
Fallow	4.92	0.84	2.32	4.81	1.98	5.15

4.3 Extractability Ratio

ER was and is still used as an index of N mineralized from soil organic matter fraction (He et al., 1988; Juma and Paul, 1984; Legg et al., 1971). In our study, it has been shown that microbial biomass fraction N calculated from Equation 3 is the best estimates for microbial biomass N, the biologically meaningful fraction; therefore, the ER from it would obtain the same meaning. Results showed that ER increased as adding ^{15}N in the growing season and ER ranged between 1.40-2.86 on four sampling of the four treatments. On each sampling, ER varied within 19, 21, 16 and 33% for faba bean, canola, barley and summer fallow. Juma and Paul (1984) argued that chloroform fumigation and aerobic incubation caused almost identical newly immobilized ^{15}N released. During 10 days of incubation of Gray Luvisolic soil, NO_3^- from non-fumigated soil was more enriched in ^{15}N than the NH_4^+ released by fumigation. The magnitude was around 40% (data not shown).

Based on all variables obtained through study, attempt was made to correlate microbial biomass C and the same origin N. Since the substrate carbon source and species of microorganisms were not determined, poor correlation between microbial biomass C and flush N would not be interpreted correctly. Further statistical analyses were conducted to clarify source of the flush N. Biomass C is negatively correlated to NO_3^-, NH_4^+ before fumigation, after fumigation and incubated, non-fumigated but incubated, and also the sum of these sources. The higher correlation coefficients were those for NO_3^- at time zero, mineral N at time zero, NO_3^- after CFIT, mineral N of non-fumigated but incubation one, and N flush. Variations of 94%can be explained by four components and 98% by 5 components. The first principal component, which accounts for 50% of the total variation, is highly, and positively correlated to three forms of $NO_3^-$and three forms of mineral N, but not NH_4^+. Therefore, this component is actually the associated with soil properties, original N status. The second component, accounted for 23%, is related to all three forms of NH_4^+ and flush N. This component is actually the one associated with CFIT effect and flush of NH_4^+.

Conclusion

Soil microbial biomass C responded significantly to cropping (faba bean, canola and barley) and summer fallow. Legume, faba bean, associated with higher microbial biomass may suggest the release of organic substances and utilization by microorganisms during their interaction. Flush N did not give significant difference among treatments, but incorporation of ^{15}N into microbial biomass N fraction did provided further information on incorporation of newly immobilized ^{15}N into microbial biomass. Microbial biomass activity in soil can not be estimated with one or two indices and interpretation of microbial biomass data is limited by multi-factors, which affect microorganisms in the soil both directly and indirectly. Microbial biomass N can be best estimated with NH_4^+ in fumigated less non-fumigated while fumigated only gives 1-6% higher results from our soil and cropping condition.

Electronic Supplementary Materials

This article contains supplementary materials in the following website.
http://ojs.udspub.com/index.php/aeb/rt/suppFiles/308/0

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