On the Recalculation of the Microbial Biomass Data

By

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1 Introduction

We had collected 63 soil samples from the pastures in the grazing intensity study site at the Central Grasslands Research Center, Streeter, ND, to run microbial biomass and carbon/nitrogen ratio. The work was done at the Soil and Water Lab at the NDSU Department of Soil Science. The samples were collected in 3 times in 2004. The first batch was collected during 26 June to 19 July (it lasted longer than had expected because several samples that had collected from 26 June to 6 July were exposed at room temperature over a weekend due to a miss understanding in arrangements). The second batch of samples was collected from 30-31 of July. The third batch was collected from 16-23 September. Each sample contained a mixture of about 10-15 sub-samples drilled from a transect of about 20-30 m long from a silty range site under particular grazing treatment (non-grazing, moderate, or heavy grazing). The soil was drilled to a depth of 20 cm using a 2.5 diameter soil sampler. Each sample had about 400 g of soils in fresh weight. The soils were passed through a 4 mm mess in order to remove course roots and other debris. Then the samples were stored at 4^oC for up to a week before transported to Fargo lab for analysis.

The soil microbial biomass was tested using the chloroform fumigation method.[1] The measurement is based on the flush of CO_2 and NH_4^+ in the "fumigated" sub-sample as compared to the "unfumigated" control sub-sample. The temporal flush of CO_2 and NH_4^+ from the "fumigated" soils was the result of the ruptures of the microbe's cell membrane systems when the fumigant fully distributed through the soil medium. The flush of CO_2 was used to calculated microbial biomass carbon(C) content, while the flush of NH_4^+ was the basis for calculating biomass nitrogen(N) content. For the case of biomass N, the equation used is

$$B_n = (F_n - U_n)/K_n \tag{1}$$

where B_n is microbial biomass N, is the flush of NH_4^+ due to fumigation, and is the NH_4^+ mineralized from a unfumigated control. The value of K_n is the proportion of microbial N mineralized to NH_4^+ during the incubation period. For the results, because the first two batches of samples have quite a few data points with negative biomass values, only the third batch of data as reported at the CGREC 2005 Annual Report. In

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[1] Horwath W.R. and Paul E.A., 1994. Microbial Biomass. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties- Soil Science Society of America Book Series, No. 5. Soil Science Society of America, 667 S. Segoe Rd., Madison, WI 53711, USA.

Table 1: Effect of long-term cattle grazing on the soil microbial biomass and carbon/nitrogen ratio. Samples were taken from 16-23 September, 2004.

$Grazing\,intensity$	$Biomass\,C(\mu g/g)$	$Biomass\,N(\mu g/g)$	C/N	*Replicate*
$Exclsoure$	406.5^a	84.9^a	7.4^a	3
$Moderate\,grazing$	399.5^a	100.7^a	4.2^a	3
$Heavy\,grazing$	487.4^a	113.6^a	4.4^a	3
Probability	$p = 0.32$	$p = 0.67$	$p = 0.56$	

the following table the effect of grazing intensity o soil microbial biomass is presented according to the data for 16-23 September 2004.

We note from table1 that effect of grazing on soil microbial biomass is not clear. The magnitude of our September data is comparable to two other studies conducted also on grasslands.[2][3] Results of Tracey and Frank (1998) indicate that soil microbial biomass did not change with long-term grazing but the biomass activity did. The negative biomass data obtained for the July samplings could be the result of (a) a high background respiration(leading to high values of control respiration); (b) lower soil microbial biomass (leading to lower values of fumigated respiration); (c) non-uniformity of the soil properties within a single sample. A varied soil moisture content might brought problems too. However, because the samples were measured under a consistent experiment system, it is possible that useful information can be obtained from the data set by looking at the data from a slightly different way, especially by correcting for the soil moisture variability.

2 A modified method of calculation

As indicated in Equation 1, the difference between F_n and U_n is the basis for calculating biomass. Under some situations (such as when soil microbial biomass is low, or when the background respiration is high, or both), this difference can easily be negative, especially when soil properties within each of the samples are not uniform (i.e., the sub-sample used for the unfumigated "control" run happened to be different from that used for the fumigated run). This non-uniformity might very likely exist in our samples, because, in order to obtain a sample representing the typical condition for a particular pasture, each of the samples we sent to Fargo actually was the composite sample of about 10-15 sub-samples collected from a 20-30 m transect. Although we passed the soils through a 4 mm mesh also the samples were mixed before laboratory analysis, it is possible that the soils were still not uniform in terms of the distribution

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[2] Tracy B.F. and Frank D.A., 1998. Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: yellowstone National Park. Oecologia. 114: 556-562.

[3] Lovell R.D. and Javis S.C., 1998. Soil microbial biomass and activity in soil from different grassland management treatments stored under controlled conditions. Soil Biol. Biochem. 30: 2077-2085.

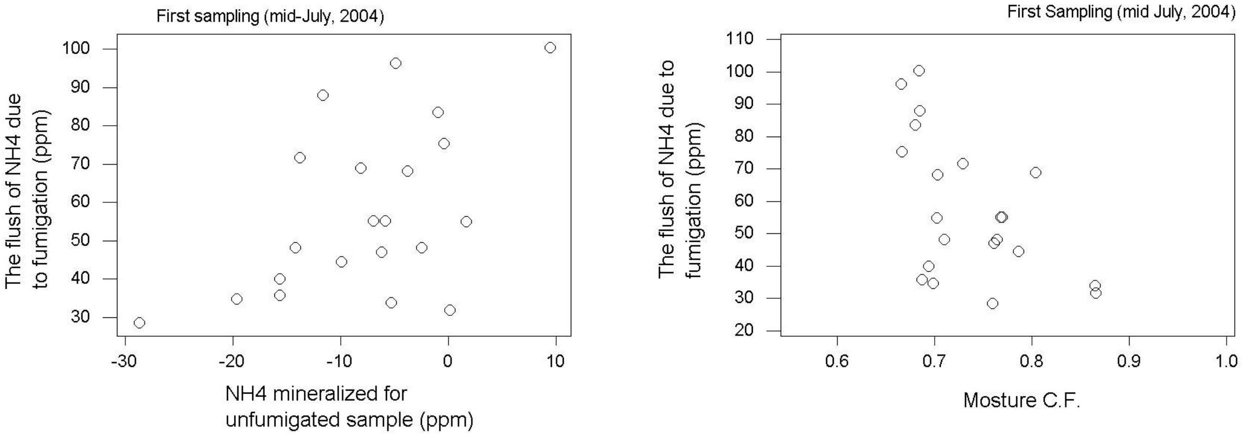

Figure 1: *a. Relationship between the fumigated and the un-fumigated* NH_4^+ *mineralized from soil sample collected in from 26 June to 19 July, 2004 (left).; b. Relationship between fumigated* NH_4^+ *mineralized and soil moisture content, also for samples collected from 26 June to 19 July, 2004 (right).*

of microbes in the soil particles. This would make relationship between F_n and U_n more scattered (Figure 1). In addition, as shown in Figure 1, F_n is strongly related with w, the moisture content of the soil samples. This is understandable because the moisture content determines the porosity for the soils, which in turn will influence the respiration rate (recall that F_n is the flush of respiration following the ruptures of the cell membrane systems by the application of the fumigant). Thus we argue that soil moisture content should be reported, or corrected explicitly, along with the F_n data. In the following, we propose a simple method for dealing with this problem. First we write F_n as a function of both U_n and w, we have

$$F_n(U_n, w) = A\,U_n^B\,w^C \tag{2}$$

where A, B and C are constants to be determined from experimental data using regression analysis. This particular form of equation was used primarily because it is a very simple nonlinear equation convertible to the linear form through logarithmic transformations, besides Qi et al (2002)[4] had used a similar form of equation to describe soil respiration as a function of soil temperature and moisture content . After Equation 2 is log-transformed (because the log function monotonously increases with the increase of the independent variable, the negative numbers in either the dependent or the independent variables can be transformed to positive ones before using the regression analysis, and change back to the original data after regression is done) and fitted with the experimental data, we can find a value F_n^*, the F_n value corresponding to U_n^* and w^*, the means of U_n and w, respectively, according to the measured data. With the data of F_n^* and U_n^*, we then use Equation 1 to calculate biomass as

$$B_n = (F_n^* - U_n^*)/K_n \tag{3}$$

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[4] Qi Y., Xu M. and Wu J., 2002. Temperature sensitivity of soil respiration and its effects on ecosystem carbon budget: nonlinearity begets surprises. Ecological Modeling. 153: 131-142.

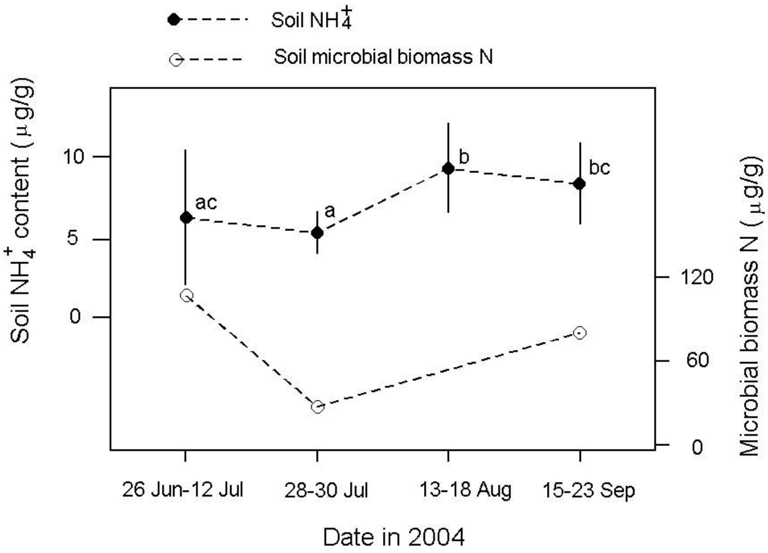

Figure 2: *Soil mineral nitrogen and microbial biomass N for the grazing intensity study site at the CGREC for 3 sampling periods in 2004. The mineral nitrogen data are from filed measurements, and the biomass data are based on our modified method of calculation. Data of* NH_4^+ *between two periods are not statistically different if they are labeled with a common letter (a, b, or c).*

The soil microbial biomass obtained from Equation 3 is the one with soil moisture content corrected. The difference between Equation 3 and Equation 1 is that the former will have to be based on data from a series of samples, while the latter will only use the data from a single sample. Considering the great variability in soil properties from natural ecosystems, we believe the modified method for calculating soil microbial biomass N, as proposed here, should be useful. In our case, we may have to pool the data set from the same batch of sampling so as to use Equations 2 and 3 to calculate the average amount of microbial biomass from our grazing pastures for each of the sampling periods.

The results are present in Table 2. According to this calculation, the soil microbial biomass N had a depression in late July, compared with the values from the late June

Table 2: Averaged soil microbial biomass nitrogen contents for the 3 sampling periods in 2004 (with the data from the 3 grazing treatments pooled). The calculation was done using Equations 2 and 3. The data points used are 21 ($n = 21$ for the 3 sampling periods). Also shown are the coefficients of determination (R^2) and the p values. The common soil moisture content of 0.77 was used throughout for the 3 sampling periods.

Date	*Equation*	R^2	p	U_n^**(ppm)*	$B_n(\mu g/g)$
$26\,Jun - 7\,Jul$	$F_n = 11.36 \times (U_n + 30)^{0.268} \times w^{-2.46}$	0.53	0.001	−7.74	106.24
$30 - 31\,Jul$	$F_n = 0.278 \times U_n^{1.09} \times w^{-7.93} - 1.5$	0.26	0.069	9.41	26.9
$16 - 23\,Sep$	$F_n = 19.30 \times U_n^{0.013} \times w^{-3.25}$	0.0.57	< 0.001	2.7	79.65

to mid-July and the one from mid-September. As shown in Figure 2, the measured soil mineral NH_4^+ seemed to have a depression in the late July also, though not statistically different from the previous (late June to mid-July) period. It is possible that at this time of the year (late July), the soluble soil nitrogen happened to be low and this might be a contributable factor for the low biomass as shown in Figure 2. The high mineral NH_4^+ for mid-August and mid-September may mainly be the result of the release of soluble nitrogen from the decay of plant roots. When compared with another report (Tracy and Frank, 1998) obtained from the Yellow Stone National Park (which presented a range of soil microbial N from less 100 to 800 μg/g), the data as presented in Table 2 are lower. Our averaged biomass N is 70.93 μg/g dry soil. If a C/N ratio of 4.3(from Table 1)is used, this corresponds to a soil microbial C of about 305 μg/g dry soil. This data of soil microbial C and N can be converted to the unit ground basis by using the bulk density of the soil. We originally had expected more from this measurements. Now it turns out that we can only obtain an averaged estimation for our grazing grasslands and use it in our ecosystem model.