

## Determination of Microbial Biomass and Nitrogen Mineralization following Rewetting of Dried Soil

A. J. Franzluebbers,\* R. L. Haney, F. M. Hons, and D. A. Zuberer

### ABSTRACT

Routine soil testing procedures that are rapid and precise are needed to evaluate agricultural surface soils for their potential to mineralize C and N. Our objectives were to determine the optimum preincubation time after rewetting of dried soil for estimating soil microbial biomass (SMB) and to identify a quick, reliable biochemical predictor of soil N mineralization potential. Biochemical determinations of SMB were screened on a Weswood silty clay loam (fine, mixed, thermic Fluventic Ustochrept) having five levels of soil organic C (SOC) as a result of long-term management. Determinations used (i) field-moist soil and (ii) soil that was air dried, rewetted, and preincubated for 0.2, 1, 3, 6, 10, and 15 d. Biochemical determinations included arginine ammonification, substrate-induced respiration (SIR), cumulative C and net N mineralization, and SMBC using the chloroform fumigation-incubation (CFI) method. Preincubation periods of 1 and 15 d prior to fumigation gave estimates of SMBC using CFI most similar to those determined on field-moist soil. Arginine ammonification and SIR determinations on dried soil were highly variable, making longer preincubation periods necessary. Carbon mineralization during all preincubation periods was highly correlated to (i) SMBC using CFI determined on field-moist and dried soil with all preincubation periods and (ii) net N mineralization during 21 d for the Weswood soil, as well as for seven additional soil series each having five to eight levels of SOC. The CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil is recommended for rapid estimation of SMBC and potential N mineralization because of its simplicity and precision.

THE IMPORTANCE of soil microorganisms to soil fertility is recognized, but rapid, accurate soil testing procedures that reflect potential C and N mineralization have not been routinely adopted (Keeney, 1982). A valid index of soil N availability that is simple, rapid, and reproducible may preclude the use of a biological method despite its importance, because of the long time period required to estimate the relatively small amount of mineralized N due to microbial activity. Incubations lasting 1 to 2 wk for determination of mineral N accumulation are considered too time-consuming for adoption by routine soil testing programs.

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The N-supplying potential of agricultural soils has been related to SMB and its activity (Carter and Rennie, 1982; Doran, 1987; Franzluebbers et al., 1994a). Measurement of SMB is sensitive to changes in the active fraction of SOM (Powlson et al., 1987; Anderson and Domsch, 1989) and therefore, should provide insight into the potential of soils to mineralize N. The most commonly used method for estimating SMB is CFI, although field-moist soil and a 10-d incubation are needed (Jenkinson and Ladd, 1981; Nannipieri et al., 1990; Parkinson and Coleman, 1991), which limit its adoption by soil testing programs. Several rapid methods for estimating SMB and its activity (i.e., C and N mineralization) have been developed during the past few decades including SIR (Anderson and Domsch, 1978) and AA (Alef and Kleiner, 1986), which require only 1 to 6 h of incubation, but as described, also require use of field-moist soil.

Soil testing protocol normally requires that dried soil be used because samples collected by producers and soil testing services are shipped to the soil testing facility, which may take several days, thereby altering the biochemical status if kept moist. We hypothesized that dried, rewetted, and preincubated soil could be used to obtain an estimate of SMB. The optimum preincubation period for estimating SMB and mineralizable N, therefore, needs to be established.

Our objectives were to: (i) evaluate the feasibility of using dried and preincubated soil for measurement of AA, SIR, cumulative C and net N mineralization, and SMBC using CFI and (ii) determine the optimum time of preincubation for these biochemical estimates.

### MATERIALS AND METHODS

Five soil samples with SMBC levels ranging from 279 to 1260 mg kg<sup>-1</sup> soil (Table 1) were collected shortly after planting wheat (*Triticum aestivum* L.) in November 1991 from a long-term field experiment established on a Weswood silty clay loam in 1982 (Table 2). Fifteen soil cores (19 mm diam.) per 4 by 12.2 m plot were collected and composited from three replications of the treatments listed in Table 1. Field-moist

**Abbreviations:** AA, arginine ammonification; BSR, basal soil respiration; CFI, chloroform fumigation-incubation; SIR, substrate-induced respiration; SMB, soil microbial biomass; SMBC, soil microbial biomass carbon; SOC, soil organic carbon; SOM, soil organic matter.

Table 1. Soil properties of the five Weswood samples determined on field-moist soil (Franzluebbers et al. 1995a).

| Sample†        | Tillage‡ | Crop sequences§   | Depth   | Soil property¶     |     |      |                                     |                                     |     |     |
|----------------|----------|-------------------|---------|--------------------|-----|------|-------------------------------------|-------------------------------------|-----|-----|
|                |          |                   |         | SOC                | TKN | SMBC | BSR                                 | NMIN                                | SIR | AA  |
|                |          |                   | mm      | g kg <sup>-1</sup> |     |      | mg kg <sup>-1</sup> d <sup>-1</sup> | mg kg <sup>-1</sup> h <sup>-1</sup> |     |     |
| VH             | NT       | Wheat/soybean     | 0-50    | 16.8               | 1.7 | 1.26 | 25.6                                | 2.5                                 | 4.3 | 0.9 |
| H              | NT       | Wheat/soy-sorghum | 0-50    | 15.5               | 1.6 | 1.00 | 19.6                                | 1.9                                 | 2.5 | 1.2 |
| M              | NT       | Wheat             | 0-50    | 11.6               | 1.2 | 0.75 | 17.2                                | 2.4                                 | 2.6 | 1.3 |
| L              | CT       | Wheat/soybean     | 50-125  | 8.3                | 1.0 | 0.48 | 9.8                                 | 1.0                                 | 3.4 | 0.5 |
| VL             | NT       | Wheat             | 125-200 | 7.4                | 0.8 | 0.28 | 7.7                                 | 0.8                                 | 1.8 | 0.4 |
| Standard error |          |                   |         | 0.5                | 0.1 | 0.04 | 1.2                                 | 0.4                                 | 1.0 | 0.4 |

† Organic matter content: VH = very high, H = high, M = medium, L = low, VL = very low.

‡ Tillage regimes are NT = no tillage and CT = conventional tillage.

§ Crops are sorghum [*Sorghum bicolor* (L.) Moench.], soybean [*Glycine max* (L.) Merr.], and wheat (*Triticum aestivum* L.).

¶ Soil properties are SOC = soil organic carbon, TKN = total Kjeldahl nitrogen, SMBC = soil microbial biomass carbon, BSR = basal soil respiration, NMIN = nitrogen mineralization, SIR = substrate-induced respiration, and AA = arginine ammonification.

samples were sieved to pass 5 mm and stored at 4°C for 59 to 81 d for determination of chemical and biological properties of field-moist soil (Franzluebbers et al., 1995b). A portion of the soil was air dried at room temperature during a 2-d period, the replications combined, sieved to pass a 2-mm screen, and stored in plastic bags. Analyses of field-moist soil were performed in the same manner as those of dried soil, except when noted otherwise.

For determination of AA (Alef and Kleiner, 1986), SIR (Anderson and Domsch, 1978), and net N mineralization, quadruplicate subsamples of 10 g of soil each were placed in 70-mL Pyrex screw-top tubes, 0.3 kg water kg<sup>-1</sup> soil (approximately -30 J kg<sup>-1</sup> soil) was added, and samples were preincubated for 0.2, 1, 3, 6, 10, or 15 d at 25°C. After the respective preincubation periods, 1 mL of arginine (120 mg kg<sup>-1</sup> soil) plus glucose (200 mg kg<sup>-1</sup> soil) solution was added to the surface of the soil in two of the tubes. To the other two tubes, 1 mL of a solution containing only glucose was added to the surface of the soil to serve as a control without addition of organic N. Since little change in respiratory activity occurs during the first 3 h after C and N addition (Smith et al., 1985), glucose addition was not expected to cause significant N immobilization during the first 3 h. After incubation for 3 h at 25°C, tubes were frozen and stored at -20°C prior to extraction for mineral N with 40 mL of 2 M KCl. The filtered extract was analyzed for NH<sub>4</sub>-N and NO<sub>3</sub>-N concentrations using autoanalyzer techniques with a salicylic acid modification

of the indophenol blue and Cd reduction methods, respectively (Bundy and Meisinger, 1994). For determination of AA, the size of SMB was related to the increase in NH<sub>4</sub>-N concentration with the addition of arginine and glucose minus the NH<sub>4</sub>-N concentration with glucose only.

Substrate-induced respiration was determined from the CO<sub>2</sub>-C evolved from the quadruplicate samples used for AA determination. At the time 1 mL of arginine-glucose or glucose only solution was added, 3 mL of 0.5 M KOH was dispensed into a 4-mL plastic vial and suspended ≈ 5 cm above the soil. The CO<sub>2</sub>-C absorbed in the alkali was determined by titration with 0.15 M HCl after addition of BaCl<sub>2</sub> (Anderson, 1982). No difference in respiratory response was observed between duplicate samples receiving arginine-glucose or glucose only (Franzluebbers et al., 1995b). Gilmour and Gilmour (1985) did not find any difference in respiratory activity during 8 h of incubation of a soil with only glucose and glucose plus (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The relative size of SMB was related to the CO<sub>2</sub>-C evolved during the 3-h incubation with added substrates. There was a six- to 22-fold increase in respiration rate due to added substrates compared with BSR. We assumed that both AA and SIR procedures determined a response to added substrate due to existing SMB and that detectable growth of SMB due to substrate addition did not occur during 3 h.

Nitrogen mineralization from field-moist soil was determined from mineral N (NH<sub>4</sub>-N, NO<sub>3</sub>-N, and NO<sub>2</sub>-N) accumulation during 10 d. Net N mineralization during 15 d of

Table 2. Physical and chemical characteristics of the eight soil series.

| Soil classification   | Location           | Clay Sand pH           |     |     | Samples | SOC†        | Land management and sampling   |
|---|--------------------|------------------------|-----|-----|---------|-------------|--|
|   |                    | — g kg <sup>-1</sup> — |     |     |         |             |  |
| Bowie fine sandy loam (fine-loamy, siliceous, thermic Plinthic Paleudult) | Overton TX         | 70                     | 740 | 5.9 | 8       | 11.6 ± 6.9  | Bermudagrass [ <i>Cynodon dactylon</i> (L.) Pers.]; poultry manure applied at 0, 10, 20, and 40 g N m <sup>-2</sup> ; 0-50- and 50-100-mm soil depths            |
| Windthorst fine sandy loam (fine, mixed, thermic Udic Paleustalf)         | Stephenville TX    | 120                    | 660 | 6.5 | 8       | 13.3 ± 6.3  | Bermudagrass; cattle manure applied at 0, 10, 20, and 40 g N m <sup>-2</sup> ; 0-50- and 50-100-mm soil depths   |
| Orelia sandy clay loam (fine-loamy, mixed, hyperthermic Typic Ochraqualf) | Corpus Christi TX  | 270                    | 560 | 8.0 | 5       | 7.1 ± 1.2   | Maize ( <i>Zea mays</i> L.); conventional disk, mixed, moldboard, and no tillage; 0-50-, 50-125-, and 125-200-mm soil depths                                     |
| Pullman silty clay loam (fine, mixed, thermic Torric Udic Paleustoll)     | Bushland TX        | 360                    | 130 | 6.0 | 8       | 9.9 ± 1.6   | Wheat ( <i>Triticum aestivum</i> L.) and sorghum [ <i>Sorghum bicolor</i> (L.) Moench]; stubble mulch and no tillage; 0-75-, 75-150-, and 150-300-mm soil depths |
| Weswood silty clay loam (fine-silty, mixed, thermic Fluventic Ustochrept) | College Station TX | 360                    | 110 | 8.2 | 5       | 11.9 ± 4.2  | Wheat, sorghum, and soybean [ <i>Glycine max</i> (L.) Merr.]; conventional disk and no tillage; 0-50-, 50-125-, and 125-200-mm soil depths                       |
| Burleson silty clay (fine, montmorillonitic, thermic Udic Pellustert)     | Taylor TX          | 400                    | 150 | 8.1 | 7       | 31.1 ± 15.6 | Cotton ( <i>Gossypium hirsutum</i> L.), sorghum, and bermudagrass; cattle manure feeding area; 0-100- and 100-200-mm soil depths                                 |
| Krum clay (fine, montmorillonitic, thermic Udertic Haplustoll)            | Hillsboro TX       | 440                    | 170 | 8.2 | 8       | 12.4 ± 2.1  | Cotton, sorghum, and bermudagrass; 0-100- and 100-200-mm soil depths   |
| Branyon clay (fine, montmorillonitic, thermic Udic Pellustert)            | Hillsboro TX       | 450                    | 190 | 8.1 | 8       | 21.0 ± 4.7  | Cotton, sorghum, and bermudagrass; 0-100- and 100-200 mm soil depths   |

† Soil organic carbon (mean ± standard deviation) of the five to eight samples.

incubation after rewetting of dried soil was determined from the  $\text{NH}_4\text{-N}$  concentration of soil receiving glucose only plus the  $\text{NO}_3\text{-N}$  concentration of soil receiving arginine-glucose or glucose. No difference in  $\text{NO}_3\text{-N}$  concentration between subsamples receiving arginine-glucose and glucose only was observed. Nitrification of the small amount of  $\text{NH}_4$  mineralized from added arginine during the 3-h incubation was apparently not detectable. Net N mineralization was described using the nonlinear regression equation (Cabrera, 1993):

$$N_t = N_i + N_0(1 - e^{-kt})$$

where  $N_t$  = inorganic N concentration ( $\text{mg N kg}^{-1}$  soil) at time  $t$  (d),  $N_i$  = initial inorganic N concentration ( $\text{mg N kg}^{-1}$  soil),  $N_0$  = N mineralization potential ( $\text{mg N kg}^{-1}$  soil), and  $k$  = nonlinear mineralization constant ( $\text{d}^{-1}$ ).

Basal respiration of field-moist soil was determined from the  $\text{CO}_2\text{-C}$  evolved during 10 d. Cumulative C mineralization of dried soil was determined from duplicate subsamples of 20 g of soil placed in 50-mL beakers, with 0.3 kg water  $\text{kg}^{-1}$  soil added, and incubated for 0.2, 1, 3, 6, 10, or 15 d at  $25^\circ\text{C}$  in 1-L air-tight sealed jars along with 10 mL of 0.5 M KOH. The quantity of  $\text{CO}_2\text{-C}$  evolved was determined by titration with 0.5 M HCl. Cumulative C mineralization was described using the nonlinear regression equation (Santruckova et al., 1993):

$$C_t = C_f(1 - e^{-kt}) + \text{BSR } t$$

where  $C_t$  = C mineralization ( $\text{mg C kg}^{-1}$  soil) at time  $t$  (d),  $C_f$  = C mineralization potential of the flush after rewetting of dried soil ( $\text{mg C kg}^{-1}$  soil),  $k$  = nonlinear mineralization constant ( $\text{d}^{-1}$ ), and BSR = basal soil respiration ( $\text{mg C kg}^{-1}$  soil).

Soil microbial biomass C was determined at 0.2, 1, 3, 6, 10, and 15 d after rewetting of dried soil from the duplicate samples that were previously used for cumulative C mineralization by exposing soil to alcohol-free chloroform vapor for 24 h (Jenkinson and Powlson, 1976). Following removal of vapors by evacuation, samples were incubated in 1-L air-tight sealed jars along with 10 mL of 0.5 M KOH for 10 d at  $25^\circ\text{C}$ . The  $\text{CO}_2\text{-C}$  evolved during the 10-d incubation following fumigation without subtraction of a control was divided by an efficiency factor of 0.41 (Voroney and Paul, 1984). Soil microbial biomass C calculated with subtraction of a 10-d control sample was also evaluated.

Air-dried soil that was further ground to pass a 0.5-mm screen was analyzed for SOC with the modified Mebius method in digestion tubes and heating at  $150^\circ\text{C}$  for 0.5 h (Nelson and Sommers, 1982) and total Kjeldahl N (Gallaher et al., 1976).

Soil samples of seven additional soil series (Table 2) with five to eight levels of SOM within each soil series were collected during 1993 and 1994, air dried, and sieved to pass 5 mm. Within each soil series, SOM content differed due to cropping history, tillage management, manure application, and/or sampling depth (Table 2). These 52 additional samples were analyzed for SOC, SMBC using CFI, and cumulative C and net N mineralization as previously described, except for the following modifications. Soil water content was adjusted to  $\approx 0.03$  MPa (i.e., 0.075  $\text{kg kg}^{-1}$  for Bowie fine sandy loam, 0.125  $\text{kg kg}^{-1}$  for Windthorst fine sandy loam, 0.25  $\text{kg kg}^{-1}$  for Orelia sandy clay loam, 0.325  $\text{kg kg}^{-1}$  for Pullman silty clay loam, 0.40  $\text{kg kg}^{-1}$  for Burleson silty clay, 0.425  $\text{kg kg}^{-1}$  for Krum clay, and 0.45  $\text{kg kg}^{-1}$  for Branyon clay). Cumulative C and net N mineralization were determined from 40-g subsamples for Bowie and Windthorst soils at 1, 3, 10, 20, and 30 d of incubation and from 20-g subsamples for Orelia, Pullman, Burleson, Krum, and Branyon soils at 1, 4, 10, and 27 d of incubation. Inorganic N was determined at 0, 10, 20, and 30

d for Bowie and Windthorst soils and at 0, 4, 10, and 27 d for Orelia, Pullman, Burleson, Krum, and Branyon soils after oven drying ( $60^\circ\text{C}$ , 24 h). Cumulative C and net N mineralization were described using nonlinear regression as described above. Regression equations were used to predict cumulative C and net N mineralization at 21 d of incubation for all soils. Soil microbial biomass C was determined at 10 d of preincubation.

Biochemical estimates from each of the aforementioned methods for each preincubation period were tested for their correlation to SMBC using CFI and BSR of field-moist Westwood soil using SAS (SAS Institute, 1990). Soil microbial biomass using CFI and BSR from field-moist soil were assumed to be the most reliable methods currently available to estimate SMB size and potential activity, respectively. Significance is at  $P \leq 0.01$  unless otherwise indicated. Regression of biochemical estimates on the  $\text{CO}_2\text{-C}$  evolved during the first day after rewetting was performed with the general linear model procedure of SAS for each soil series separately and together. Soil pH and clay content were tested for significance in the pooled analyses as covariates.

## RESULTS AND DISCUSSION

### Preincubation Period for Biochemical Determinations

Soil microbial biomass with the CFI method, BSR, and net N mineralization determined from field-moist soil were highly related to SOC and TKN, but AA and SIR were not (Table 1). Only with a larger data set (Franzluebbbers et al., 1995b) were estimates with AA and SIR correlated to SOC and TKN. The coefficient of variation for analysis on field-moist soil in this study was 80% for AA, 62% for SIR, 42% for net N mineralization, 13% for BSR, and 8% for SMBC. Soil organic C and total Kjeldahl N had coefficients of variation of 7%. Inherent soil and methodological variability appeared to be a major limitation in describing the relatively small response in  $\text{NH}_4\text{-N}$  mineralization with AA and  $\text{CO}_2\text{-C}$  evolution with SIR during the 3-h incubation.

Estimates of SMB using AA on rewetted soil were correlated with SMBC using CFI on field-moist soil for most preincubation periods (Table 3). Although relationships of AA with SMBC using CFI were statistically significant, they were not strong enough ( $r^2 > 0.67$ ) to be used to predict SMB. Depending on preincubation period, correlations were positive and negative. Lack of consistency among AA and other methods with respect to preincubation time further indicates unreliability for prediction of SMB. Despite methodological rapidity, AA determined on rewetted soil cannot be recommended for routine soil testing unless further modifications are made to reduce variability.

Estimates of SMB using SIR were best correlated with SMBC using CFI on field-moist soil at 1 and 15 d of preincubation (Table 3). Correlations were variable at other preincubation periods, indicating that this method may not be reliable for rewetted soil without an extended preincubation. Long preincubation would exclude this method as a rapid soil testing procedure. The reliability of SIR and AA for rewetted soil appears to be question-

**Table 3. Correlation coefficients relating biochemical estimates determined on dried Weswood soil preincubated for 0.2, 1, 3, 6, 10, and 15 d to soil microbial biomass C using chloroform fumigation-incubation on field-moist Weswood soil ( $n = 5$ ).**

| Determination‡ | Condition§      | Correlation coefficient |
|----------------|-----------------|-------------------------|
| SOC            | dried           | 0.965***                |
| TKN            | dried           | 0.976***                |
| AA             | field-moist     | 0.428                   |
| AA             | D/W, 0.2 d      | 0.629†                  |
| AA             | D/W, 1 d        | 0.788**                 |
| AA             | D/W, 3 d        | -0.694†                 |
| AA             | D/W, 6 d        | -0.576†                 |
| AA             | D/W, 10 d       | 0.636†                  |
| AA             | D/W, 15 d       | 0.710†                  |
| SIR            | field-moist     | 0.330                   |
| SIR            | D/W, 0.2 d      | 0.111                   |
| SIR            | D/W, 1 d        | 0.874***                |
| SIR            | D/W, 3 d        | 0.719***                |
| SIR            | D/W, 6 d        | 0.521†                  |
| SIR            | D/W, 10 d       | 0.703**                 |
| SIR            | D/W, 15 d       | 0.933***                |
| CMIN           | f-moist, 0-10 d | 0.960***                |
| CMIN           | D/W, 0-0.2 d    | 0.575                   |
| CMIN           | D/W, 0-1 d      | 0.956***                |
| CMIN           | D/W, 1-3 d      | 0.974***                |
| CMIN           | D/W, 3-6 d      | 0.990***                |
| CMIN           | D/W, 6-10 d     | 0.954***                |
| CMIN           | D/W, 10-15 d    | 0.949***                |
| NMIN           | f-moist, 0-10 d | 0.699**                 |
| NMIN           | D/W, 0.2 d      | 0.934***                |
| NMIN           | D/W, 1 d        | 0.911***                |
| NMIN           | D/W, 3 d        | 0.917***                |
| NMIN           | D/W, 6 d        | 0.806**                 |
| NMIN           | D/W, 10 d       | 0.948***                |
| NMIN           | D/W, 15 d       | 0.960***                |
| CFI            | D/W, 0.2 d      | 0.981***                |
| CFI            | D/W, 1 d        | 0.984***                |
| CFI            | D/W, 3 d        | 0.975***                |
| CFI            | D/W, 6 d        | 0.975***                |
| CFI            | D/W, 10 d       | 0.975***                |
| CFI            | D/W, 15 d       | 0.938***                |

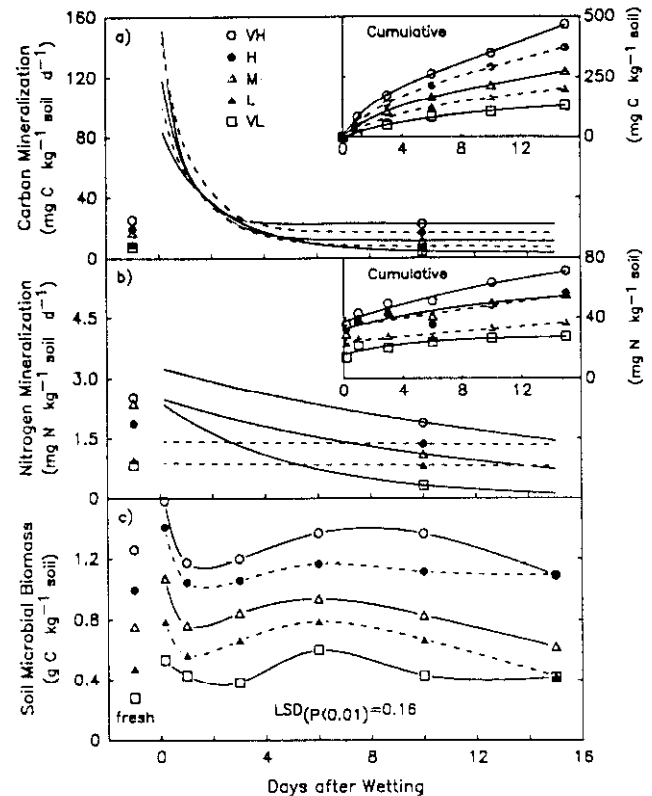
†, \*\*, and \*\*\* Significant at  $P \leq 0.1$ ,  $0.01$ , and  $0.001$ , respectively.

‡ Determinations were SOC = soil organic carbon, CMIN = C mineralization, AA = arginine ammonification, CFI = chloroform fumigation-incubation, TKN = total Kjeldahl nitrogen, NMIN = net nitrogen mineralization, and SIR = substrate-induced respiration.

§ Condition of soils were field-moist (without drying) and D/W (air-dried and subsequently rewetted with a preincubation period between 0.2 and 15 d).

able, with coefficients of variation for both assays ranging from 70 to >100% (data not shown).

Carbon mineralization and SMBC using CFI at all preincubation periods, except C mineralization during 0.2 d, were highly related to SMBC using CFI on field-moist soil (Table 3). Soil microbial biomass C using CFI on rewetted soil compared with field-moist soil was an average of 43% greater at 0.2 d of preincubation, 6% greater at 1 d ( $P \leq 0.1$ ), 10% greater at 3 d, 29% greater at 6 d, 17% greater at 10 d, and not different at 15 d (Fig. 1c). The overestimation of SMBC at 0.2 d of preincubation, expressed as a percentage of SMBC using field-moist soil, increased with decreasing level of SMBC (Fig. 1c). The flush of  $\text{CO}_2\text{-C}$  during the first 3 d after rewetting (Fig. 1a) probably caused this overestimation in SMBC. Soil microbial biomass C determined immediately after rewetting of dried soil resulted in values 25% greater than from field-moist, undisturbed soil (Shen et al., 1987). Estimation of SMBC using CFI



**Fig. 1. (a) Carbon mineralization, (b) net N mineralization, and (c) soil microbial biomass C as a function of time after rewetting of dried soil. Weswood soil organic matter content: very high (VH), high (H), medium (M), low (L), and very low (VL). Values for field-moist soil are indicated by symbols placed before 0 d of incubation. Actual data for C and N mineralization are depicted in the cumulative subpanels. The rate of C and N mineralization was calculated from cumulative C and N mineralization. Symbols at 10 d are for identification of rate curves for C and N mineralization.**

with subtraction of a 10-d control value on field-moist soil was highly related to the same procedure without subtraction of a control ( $r = 0.99$ ). A similarly close correlation ( $r = 0.99$ ) has been observed previously (Jenkinson and Powlson, 1976). Estimates of SMBC using CFI on rewetted soil with a control subtracted averaged 49% greater than on field-moist soil at 0.2 d of preincubation, 10% less at 1 d, 24% greater at 3 d, 76% greater at 6 d, 58% greater at 10 d, and 18% greater at 15 d.

We recommend that soils be preincubated for  $\geq 10$  d in order to stabilize the SMB following disturbance caused by drying and rewetting, because 90% of the flush of  $\text{CO}_2\text{-C}$  due to rewetting of dried soil was evolved within 4 to 10 d. Samples with lower SOM took a longer time to reach a steady-state BSR. However, the length of the preincubation period mattered little in separating relative differences in SMBC using CFI among samples with different SOM levels. This is of importance since the determination of SMBC using CFI and other methods should be considered a relative rather than an absolute estimate.

### A Rapid Test for Nitrogen Mineralization

Carbon mineralization from Weswood soil during the first day after rewetting was highly related to cumulative

Table 4. Regression equations relating the CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil to cumulative C and net N mineralization during 21 d (CMIN<sub>0-21 d</sub> and NMIN<sub>0-21 d</sub>, respectively) and to soil microbial biomass C (SMBC) for eight soils from Texas. Actual data for CMIN<sub>0-1 d</sub>, NMIN<sub>0-21 d</sub>, and SMBC are in Fig. 2.

| Soil           | CMIN <sub>0-21 d</sub> =<br>a + b(CMIN <sub>0-1 d</sub> ) |              |                | NMIN <sub>0-21 d</sub> =<br>a + b(CMIN <sub>0-1 d</sub> ) |              |                | SMBC =<br>a + b(CMIN <sub>0-1 d</sub> ) |              |                |
|----------------|---|--------------|----------------|---|--------------|----------------|---|--------------|----------------|
|                | Intercept<br>(a)  | Slope<br>(b) | r <sup>2</sup> | Intercept<br>(a)  | Slope<br>(b) | r <sup>2</sup> | Intercept<br>(a)                        | Slope<br>(b) | r <sup>2</sup> |
| Bowie fsl      | -22   | 11.0***      | 0.94           | -0.3  | 1.21***      | 0.89           | -56                                     | 21.3***      | 0.92           |
| Windthorst fsl | -86   | 10.4***      | 0.95           | 10.0  | 0.59†        | 0.62           | -205                                    | 17.4***      | 0.85           |
| Orelia scl     | -25   | 5.8**        | 0.93           | 12.4†   | 0.27         | 0.62           | 24                                      | 15.8**       | 0.95           |
| Pullman sicl   | -49   | 6.0***       | 0.94           | 4.0   | 0.60**       | 0.66           | 2                                       | 20.4**       | 0.74           |
| Weswood sicl   | 7   | 7.5***       | 0.99           | 2.5   | 0.46***      | 0.99           | 149**                                   | 15.3***      | 0.99           |
| Burleson sic   | -74†  | 6.3***       | 0.99           | -10.0   | 1.04***      | 0.91           | -126                                    | 25.2***      | 0.97           |
| Krum c         | -15   | 7.7***       | 0.90           | 19.1**  | 0.32**       | 0.78           | 601†                                    | 18.3**       | 0.79           |
| Branyon c      | -42   | 6.2***       | 0.97           | 6.8   | 0.66***      | 0.91           | 885***                                  | 12.4***      | 0.91           |

†, \*\*, and \*\*\* Significant at  $P \leq 0.1$ , 0.01, and 0.001, respectively.

C and net N mineralization from 0 to 15 d (Fig. 1a and 1b):

$$\text{CMIN}_{0-15 d} = 22 + 5.7(\text{CMIN}_{0-1 d}), r^2 = 0.99 \text{ and}$$

$$\text{NMIN}_{0-15 d} = 3.4 + 0.35(\text{CMIN}_{0-1 d}), r^2 = 0.87$$

Therefore, the flush of CO<sub>2</sub>-C following rewetting of dried soil may be a reliable method to estimate the potential of a soil to mineralize C and N. Prediction of C mineralized during 15 d by C mineralized during 1 d is supported by previous observations, in which relative differences in cumulative C mineralization among soils early in incubations are often maintained throughout extended incubations (Honeycutt et al., 1988; Franzluebers et al., 1995c).

Carbon mineralization during the first day after rewetting of dried soil as a predictor of net N mineralization was evaluated further with seven additional soils from Texas (Table 2). The CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil was highly related in most cases to the cumulative C and net N mineralization during 21 d and to SMBC (Table 4). Differences in slopes among soils between CMIN<sub>0-1 d</sub> and CMIN<sub>0-21 d</sub>, NMIN<sub>0-21 d</sub>, and SMBC were significant, although these differences were not related to soil texture or pH in an analysis of covariance. Differences in slopes among soils could be attributed to differences in intercepts among soil series, which affected slope estimates. Despite these differences among soils, a common relationship between the CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil and SMBC ( $r^2 = 0.87$ ) and net N mineralization during 21 d ( $r^2 = 0.85$ ) was observed (Fig. 2).

We attempted to corroborate the relationship between CO<sub>2</sub>-C evolved during the first day after rewetting and net N mineralization with several published reports (Table 5). The CO<sub>2</sub>-C evolved during the first day after rewetting predicted net N mineralization with a standard deviation of  $\pm 8 \text{ mg N kg}^{-1} \text{ soil}$ . Six of 15 observations were predicted  $\pm 5 \text{ mg N kg}^{-1} \text{ soil}$  and 13 of 15 observations were predicted  $\pm 10 \text{ mg N kg}^{-1} \text{ soil}$ .

Prediction of SMBC and net N mineralization from CO<sub>2</sub>-C evolved during the first day after drying has a firm theoretical basis. Drying soil kills part of the SMB (Jenkinson, 1966), as well as rendering some nonliving SOM mineralizable due to chemical and physical disturbance (Kieft et al., 1987; van Gestel et al., 1991). The

flush of activity during the first day, therefore, reflects the contribution of both the SMB and active SOM pools that are readily mineralizable. Prediction of net N mineralization from CO<sub>2</sub> evolution has been suggested for plant residues added to soil (Gilmour et al., 1985). The relationship between C and N mineralization during 30 d differed among plant residues due to the initial C/N ratio of the residue, as well as the C/N ratio of the residue remaining after decomposition of the rapidly mineralizable fraction (Gilmour et al., 1985). The overall C/N ratio of most soils is relatively stable between 8 and 12 (Alexander, 1991), therefore variation in the relationship between C and N mineralization among soils would be more a function of the active fraction of SOM, including the SMB, its metabolic by-products, and recently intro-

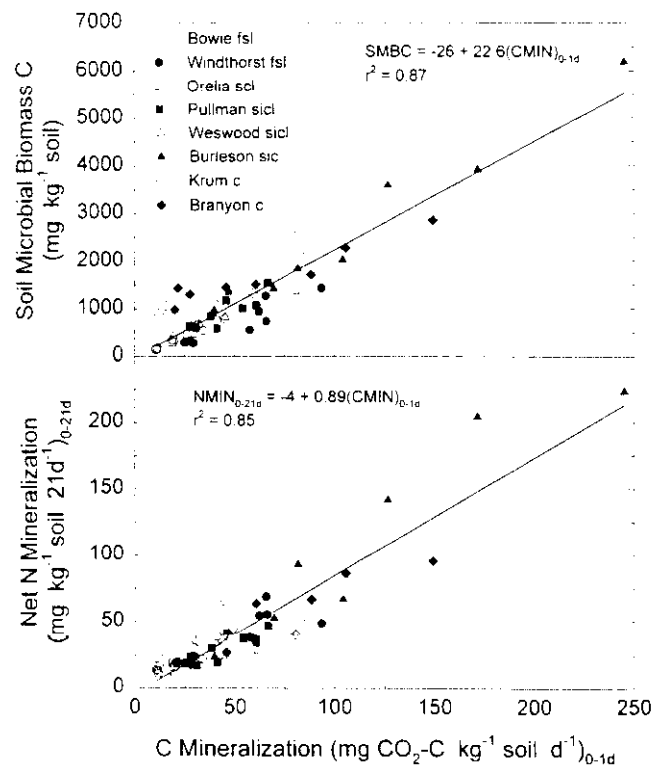


Fig. 2. Relationship of soil microbial biomass C and net N mineralization during 21 d to the CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil from eight soils in Texas. Dotted lines represent 95% confidence interval.

Table 5. Prediction of net N mineralization during 21 d (NMIN<sub>0-21 d</sub>) from previously published data based as a function of the predicted CO<sub>2</sub>-C evolved during the first day after rewetting (CMIN<sub>0-1 d</sub>) [NMIN<sub>0-21 d</sub> = -4 + 0.89(CMIN<sub>0-1 d</sub>)].†

| Reference and location                    | Soil texture and pH  | mg kg <sup>-1</sup> soil |                        |                                  | Experimental conditions                                     |
|---|----------------------|--------------------------|------------------------|----------------------------------|---|
|   |                      | CMIN <sub>0-1 d</sub>    | NMIN <sub>0-21 d</sub> | Predicted NMIN <sub>0-21 d</sub> |   |
| Bowman et al. (1990), Colorado            | sandy loam, 6.8      | 58.8                     | 41.4                   | 48.3                             | 30°C, 0-21 d; prairie, cultivated                           |
|   |                      | 12.7                     | 12.4                   | 7.3                              |   |
|   |                      | 15.9                     | 14.2                   | 10.2                             |   |
|   |                      | 18.0                     | 12.0                   | 12.0                             |   |
| Boyle and Paul (1989), California         | loam, 5.4            | 12.9                     | 5.1                    | 7.4                              | 25°C, 0-11 wk;<br>0, 1.8, and 4.5 Mg sludge m <sup>-2</sup> |
|   |                      | 32.4                     | 33.2                   | 24.7                             |   |
|   |                      | 35.3                     | 36.5                   | 27.3                             |   |
|   |                      | 32.2                     | 15.8                   | 24.5                             |   |
| DeBruin et al. (1989), Mali               | loamy sand, 5.3      | 42.0                     | 17.3                   | 33.3                             | 30°C, 0-28 d  |
|   |                      | 38.7                     | 16.3                   | 30.4                             |   |
| Hadas and Portnoy (1994), Israel          | clay, 7.8            | 22.8                     | 13.8                   | 16.2                             | 28°C, 0-28 d  |
|   |                      | 16.8                     | 7.9                    | 10.9                             |   |
| Nicolardot et al. (1994), France, Belgium | silt loam, 8.3       | 8.9                      | 10.3                   | 3.9                              | 37°C, 0-28 d  |
|   |                      | 40.4                     | 22.5                   | 31.8                             |   |
| Robertson et al. (1988), Sweden           | loamy, 7.6           | 8.9                      | 10.3                   | 3.9                              | 37°C, 0-28 d  |
|   |                      | 40.4                     | 22.5                   | 31.8                             |   |
| Smith et al. (1994a), Washington          | sandy clay loam, 6.9 | 57.1                     | 51.7                   | 47.1                             | 23.5°C, 0-21 d  |
|   |                      | 29.7                     | 20.7                   | 22.4                             |   |
| Mean (n = 15)                             | silt loam (N/A)      | 29.7                     | 20.7                   | 22.4                             |   |

† CMIN and NMIN were predicted from the equations reported in Boyle and Paul (1989) and Smith et al. (1994a) and predicted with the equations [C<sub>t</sub> = C<sub>i</sub>(1 - e<sup>-kt</sup>) + BSRt; N<sub>t</sub> = N<sub>i</sub> + N<sub>0</sub>(1 - e<sup>-kt</sup>)] using data available from 0 to 28 d in all other studies.

duced organic residues. Differences in the relationships among soils presented in Table 4 were probably due to differences in active SOM, as well as chemical and physical differences that remain to be defined.

Net N mineralization was also highly related to SOC for all soils, with the relationship:

$$\text{NMIN}_{0-21 \text{ d}} = -13.9 + 3.78(\text{SOC}), r^2 = 0.87.$$

Differences in this relationship among soils were not significant (data not shown). Except for the Pullman soil, the proportion of SOC as SMBC did not influence the relationship between net N mineralization and the flush of CO<sub>2</sub>-C during the first day after rewetting, suggesting that active fractions of SOM other than the SMB probably contributed more to differences in net N mineralization among soils.

A potential limitation for using the relationship between the CO<sub>2</sub>-C evolved during the first day after rewetting and net N mineralization may be that soils sampled during a period after addition of organic material with a high C/N ratio (e.g., cereal straw or rhizodeposition products) may lead to an overestimation of the short-term N availability due to N immobilization (Franz-luebbbers et al., 1994b, 1995a). Limiting this relationship to soil samples collected only in winter and spring, as is typical for summer crops, or as late after residue incorporation as possible could alleviate this potential complication with N immobilization.

Net N mineralization during the first 2 wk after rewetting dried soil was closely related to the potentially mineralizable N during 30 wk of successive leaching-incubation (Stanford and Smith, 1972):

$$\text{NMIN}_{0-210 \text{ d}} = 39 + 4.0(\text{NMIN}_{0-14 \text{ d}}), r^2 = 0.76$$

and during 24 wk of incubation (Smith et al., 1994b):

$$\text{NMIN}_{0-168 \text{ d}} = 25 + 2.8(\text{NMIN}_{0-14 \text{ d}}), r^2 = 0.85.$$

We found that determination of CO<sub>2</sub>-C evolution under standard conditions is considerably simpler and less time consuming than periodic determination of inorganic N

from aerobic or anaerobic incubations, whether conducted with short-term destructive sampling or with long-term leaching incubations. Prediction of potential N mineralization from the CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil may be a valuable procedure for use in routine soil testing laboratories where simplicity, rapidity, and reproducibility are important criteria.

## CONCLUSIONS

Further research is needed to identify the factors controlling the differences in relationships between the CO<sub>2</sub>-C evolved during the first day after rewetting and net N mineralization among soils. However, due to its relative simplicity, rapidity, and reliability, we recommend that the quantity of CO<sub>2</sub>-C evolved during the first day after rewetting of dried soil be considered as a rapid test to estimate net N mineralization potential associated with the size and potential activity of the SMB. Relationships between the flush of CO<sub>2</sub>-C evolved during the first day after rewetting and net N mineralization for individual soil series may need to be developed to increase the precision of this technique.

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