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# Municipal solid waste compost dose effects on soil microbial biomass determined by chloroform fumigation-extraction and DNA methods

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**Abstract -** We evaluated the relationship between microbial biomass C and N ($B_C$ and $B_N$) as estimated by the chloroform fumigation-extraction (CFE) method and microbial biomass DNA concentration in a loam-clayey wheat cultivated soil. The soil received municipal solid waste compost at rates of 40 or 80 t $ha^{-1}$ and farmyard manure at 40 t $ha^{-1}$. Microbial biomasses C and N and DNA concentration showed the highest values for microorganisms counts with compost and farmyard manure at 40 t $ha^{-1}$. Compost applications at 40 t ha-1 improve the micro-organisms growth than that of 80 t $ha^{-1}$. Moreover a significant decrease of soil microbial biomass was noted after fertilisation for three years. The presence of humic acid and proteins impurities in DNA extracts; even in important level as in F-treated soil; did not affect the microbial biomass. The decrease of microbial biomass was due to heavy metals content elevation in compost at 80 t $ha^{-1}$ treated soil. Thus the highest rate of municipal solid waste compost induced the lowest ratio of biomass C to soil organic carbon and the lowest ratio of biomass N to soil organic nitrogen. There was a positive relationship between $B_C$, $B_N$ and DNA concentration. DNA concentration was significantly and positively correlated with $B_C$ and with $B_N$. However there was a negative correlation between either micro-organisms numbers and DNA concentration, or $B_C$ and $B_N$. The comparison of the two used methods DNA extraction and CFE showed the lowest coefficient of variation (cv %) with DNA extraction method. This last method can be used as an alternative method to measure the microbial biomass in amended soils.

**Key words**: municipal solid waste compost, microbial biomass C, microbial biomass N, chloroform fumigation extraction, DNA extraction.

## INTRODUCTION

The soil microbial biomass (SMB) conducts biochemical transformations in soil (Breland and Eltun, 1999). The potential influence of the SMB in a soil sample may be assessed by its amount (Anderson and Domsch, 1989). Several studies showed that the SMB varies with soil management including the farming system (Hu *et al*., 1997), fertilisation (Salinas-Garcia *et al*., 1997), organic amendments (Jedidi *et al*., 2004) and heavy metals (Garcia-Gil *et al*., 2000). Soil microbial biomass may be used as a sensitive and as an indicator of environmental changes of soil management (Bouzaiane *et al*., 2007).

Assessment of SMB can be achieved by direct methods, such as the micro-organisms counts (Paul and Johnson, 1977), or by indirect methods, such as the chloroform fumigation-extraction method (CFE) (Vance *et al*., 1987; Tate *et al*., 1988). The CFE (Vance *et al*., 1987; Brookes, 1995) has been widely used to estimate microbial biomass under different field and laboratory conditions. The microbial biomass C and N have been estimated, using CFE in both cultivated and uncultivated soils (Vong *et al*., 1990), in forest soils (Gallardo and Schlesinger, 1990), and in seasonnally dried soils (Wu and Brookes, 2005). The DNA quantification method (DNA) has been compared to the CFE method in different soils (Marstorp *et al*., 2000; Bailey *et al*., 2002; Leckie *et al*., 2004) and has been proposed as an alternative method of CFE to measure SMB (Marstorp *et al*., 2000). Direct extraction of total DNA from the soil, often results in coextraction of DNA and other soil components mainly humic substances, which negatively interfere with DNA dosage (Steffan *et al*., 1988; Trevors *et al*., 1992).

The objectives of this study were: (i) to observe the effects of municipal solid waste compost dose on the progress of microbial biomass determined by fumigation-extraction and DNA methods (ii) and to observe whether purified, extracted DNA may be an appropriate measure of total microbial biomass and an alternative method to the fumigation-extraction in municipal solid waste compost treated soil.

## MATERIALS AND METHODS

**Experimental site and soil sampling.** The study was conducted in the north of Tunisia, in a wheat (Var. Karim) cultivated plot. The climate is semi arid with annual mean temperature of 18.6 °C and of 119.15 mm precipitation. For the wheat cultivated plot, the experiment was set up in

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a random block design with four replications and an elementary plot size of 2.25 $m^2$.

Total N was determined by the Kjeldahl method as recommended by Brookes *et al*. (1985), while the organic C content was determined by dry combustion (Walkley and Black 1934). The total heavy metals (Cu, Zn, Ni, Pb, Cr and Cd) in soils were determined by an atomic absorption spectrophotometer after acid digestion (nitric acid and chloridric acid, 3/1, v/v).

Treatments used were: non-treated soil (S); a livestock farmyard manure of cow is used in this experiment at rate of 40 t $ha^{-1}$ (F); mature municipal solid waste compost (MSWC) was obtained from sorted municipal solid wastes by aerobic fermentation of 120 days in the composting plant. The annual application rates given as ton dry matter used in our case study were MSWC at rates of 40 t $ha^{-1}$ (C1) and at 80 t $ha^{-1}$ (C2). Some physico-chemical characteristics of the soil and organic amendments used in this study were summarised in Table 1.

The soil sampling was performed three years after organic matter application; in June at the end of culture and at a depth of 0-20 cm. All samples were stored at 4 °C prior to analyses.

TABLE 1 - Soil and organic amendments characteristics used

| | Soil | Compost | Farmyard manure |
|---|---|---|---|
| pH (in water) | 8.5 (0.2) | 7.9 (0.2) | 7.8 (0.30) |
| C (%) | 0.87 (0.01) | 17.50 (1.30) | 29.20 (2.40) |
| N (%) | 0.095 (0.002) | 1.800 (0.030) | 2.600 (0.090) |
| C/N | 9.15 | 9.8 | 11.4 |
| HR (%) | 8.2 | 25.8 | 7.1 |
| Clay (%) | 27 (0.8) | Na | Na |
| Silt (%) | 62 (1.4) | Na | Na |
| Sand (%) | 11 (0.5) | Na | Na |
| Cd (mg $kg^{-1}$) | 1.1 (0.03) | 2.3 (0.30) | 2.1 (0.05) |
| Pb (mg $kg^{-1}$) | 49.5 (2.3) | 80.1 (3.6) | 8.9 (0.9) |
| Cr (mg $kg^{-1}$) | 22.5 (1.1) | 78.9 (2.9) | 25.9 (2.5) |
| Ni (mg $kg^{-1}$) | 21.9 (1.8) | 90.8 (4.1) | 22.4 (1.8) |
| Cu (mg $kg^{-1}$) | 42.5 (0.3) | 337 (6.8) | 25.5 (1.3) |
| Zn (mg $kg^{-1}$) | 115.7 (2.2) | 290.2 (11.7) | 117.1 (3.1) |

C: carbon; N: nitrogen; HR: moisture; Cd: cadmium; Pb: lead; Cr: chrome; Ni: nickel; Cu: copper; Zn: zinc.
n = 3; (In brackets): standard deviation; NA: not applicable.

**The chloroform-fumigation method.** Microbial biomass C and N were analysed by the CFE method, according to Vance *et al*. (1987) and Brookes (1995), respectively.

Duplicate samples (20 g) of treated soils and non-treated were fumigated with ethanol-free $CHCl_3$ for 24 h. Fumigated and non-fumigated soil samples were extracted with 0.5 M $K_2SO_4$ (1/4, w/v). Organic C was quantified by the potassium dichromate oxidation method (Jenkinson and Powlson, 1976) and subsequent back-titration of the unreduced dichromate. The soil microbial biomass C (MBC) was estimated using the following equation (Jenkinson and Powlson, 1976):

$$MBC = CE/0.35$$

where CE was the difference between organic C extracted from fumigated and non-fumigated treated soils.

Total N in the extracts was determined according to the Kjeldahl methods as described by Brookes *et al*. (1985). The microbial biomass N was estimated using the following equation:

$$MBN = NE/0.68$$

where NE was the difference between total N extracted from fumigated and non-fumigated soils. Amounts of microbial biomass C or N were expressed (mg C or N $kg^{-1}$ dry weight) on air-dry soil basis and represent the average of three determinations.

**DNA extraction method.** DNA was extracted and purified from equivalent dry weights of each soil sample (500 mg fresh soil), using the Bio 101 Fast DNA Kit for Soil (Biogène, France), according to the manufacturer instructions. Purified DNA was quantified by spectrophotometer (Bio-RAD Smart Spec $^{TM}$ Plus, France) (Leckie *et al*., 2004). The spectrophotometric *A260 /A280* and *A260 /A230* ratios were determined to evaluate levels of protein and humic acid impurities, respectively, in the extracted DNA (Ogram *et al*., 1987; Steffan *et al*., 1988).

**Microorganisms counts.** Aliquots of 5 g of soil were used to determine the number of culturable microorganisms. Samples were plated onto 10-fold-diluted tryptic soy agar (BIO RAD, France) containing 100 µg cycloheximide $ml^{-1}$ to inhibit fungal growth. Plates were incubated at 25 °C for three days and then the numbers of colonies forming units (CFU) were counted.

**Statistical analysis.** The ANOVA analysis was carried out using the SPSS statistical program for Windows (SPSS Inc., Chicago, IL). The means were compared according to the Newman and Keuls multiple range-test. Pearson's correlation coefficients were calculated for selected parameters. All statistical analyses were performed at $P \leq 0.05$ or at $P \leq 0.01$.

## RESULTS AND DISCUSSION

### pH, total organic C and organic N

There were no significant differences ($P < 0.05$) between pH values of all soil treatments (Table 2), reflecting the buffer capacity of the soil. Similar results were obtained by Duchaufour (1997) in a clayey soil with high $CaCO_3$ content.

The total organic C (TOC) investigation showed a positive effect of organic matter amendment with either compost or farmyard manure after three years application. In fact a significant increase of 22, 48 and 245% for F-treated soil, C1-treated soil and C2-treated soil, respectively (Table 2). Similar results were obtained by Kaschl *et al*. (2002) who reported an increase in the organic matter in compost amended soil. Also Bouzaiane *et al*. (2007) noticed that the MSWC used during this study appeared loaded with organic matter and micro-organisms than the farmyard manure. Application of compost at rates of 40 t $ha^{-1}$ and 80 t $ha^{-1}$ increased the TOC and this increase is higher that with F-treated soil. Similar results were obtained by Eghball (2002); after 4 years of compost application higher organic matter content than with farmyard manure application were obtained.

TABLE 2 - pH, total organic C, microbial C ratio, organic N and microbial N ratios

| Treatments | pH | TOC (%) | $B_C$ (%) | $B_C$/TOC | $N_{org}$ (%) | $B_N$ (%) | $B_N/N_{org}$ |
|---|---|---|---|---|---|---|---|
| S | 8.57 (0.06)a | 1.10 (0.1)a | 6.51 (0.62)a | 5.9 | 0.17 (0.03)a | 4.52 (0.4)a | 26.6 |
| F | 8.63 (0.14)a | 1.35 (0.2)a | 14.30 (2.10)b | 10.5 | 0.32 (0.1)a | 12.70 (3.6)c | 39.7 |
| C1 | 8.49 (0.05)a | 1.63 (0.1)b | 22.10 (3.12)d | 13.5 | 0.33 (0.09)a | 15.70 (2.7)d | 47.6 |
| C2 | 8.60 (0.05)a | 3.88 (0.3)c | 18.20 (1.11)c | 4.77 | 0.58 (0.11)b | 9.90 (1.6)b | 17.0 |

S: non-treated soil; F: farmyard manure at 40 t $ha^{-1}$ treated soil; C1: compost at 40 t $ha^{-1}$ treated soil; C2: compost at 80 t $ha^{-1}$ treated soil; TOC: total organic C; $N_{org}$: organic N.
n = 4; (In brackets): standard deviation; within a column different letter after bracket means that the value is significantly different according to Student-Newmann-Keuls test at $P < 0.05$.

The soil organic nitrogen was also increased by fertilisation. Subsequent values of organic nitrogen were 88 and 94%, after farmyard manure and compost at 40 t $ha^{-1}$ application, respectively (Table 2). The higher value of organic nitrogen of 241% was obtained after with compost at 80 t $ha^{-1}$ application. Results showed that organic nitrogen did not differ significantly for non-treated soil, F-treated soil and C1-treated soil. However there is a significant difference between C2-treated soil and F-treated soil or between C1-treated soil and C2-treated soil.

**Amendment dose and type effects on soil microbial biomass and DNA concentration**

Microbial biomass C and N in the non-treated soil were significantly different from those of treated soils (Fig. 1). The application of either compost or farmyard manure showed an increase in $B_C$, $B_N$ and DNA concentration. The microbial biomass C and N, and DNA concentration in the C1-treated soil were significantly different from those in C2-treated soil ($P < 0.05$). It is notable that DNA concentration in C1-treated soil was higher than that in C2-treated soil (Fig. 1C). So that, the application of compost at 40 t $ha^{-1}$ enhance microorganisms growth than that of 80 t $ha^{-1}$, this result support the suggestion made by Jedidi *et al*. (2004), who used compost at 40 t $ha^{-1}$ but in laboratory studies, and recommended this level for field. Our results revealed that when compost dose increased from 40 to 80 t $ha^{-1}$, the microbial $B_C$ and $B_N$ and DNA concentration decreased significantly.

Microbial biomass C and N in compost at 40 t $ha^{-1}$ treated soil were significantly different from those obtained in F-treated soil. However, no significant differences between the microbial biomass C and N in compost-treated soil or in farmyard manure-treated soil at 40 t $ha^{-1}$ in laboratory study was found by Jedidi *et al*. (2004). Moreover, DNA concentration in compost treated soil was higher than that in the F-treated soil. These findings could be explained by the enhancement of C-retaining microbial activities in soil with the compost application than with the farmyard manure use. This could be also resulted from more C-stabilizing (or composting) micro-organisms that may be introduced into the soil with the compost application.

**Carbon and nitrogen microbial ratios**

The microbial C ratio ($B_C$ / TOC) increased after fertilisation (Table 2). In non-treated soil, the microbial ratio was 5.9, this ratio increased with farmyard manure (10.5) and with compost at 40 t $ha^{-1}$ (13.5) while decreased with compost at 80 t $ha^{-1}$ (4.77). Also Garcia-Gil *et al.* (2000) showed that microbial biomass increased with the organic amendments that contained microbial biomass in the organic residues and the addition of substrate-C, which stimulates the indigenous soil microbiota. This could be explained by the soil enrichment in humus instead of microbial C when compost is applied at elevated rates.

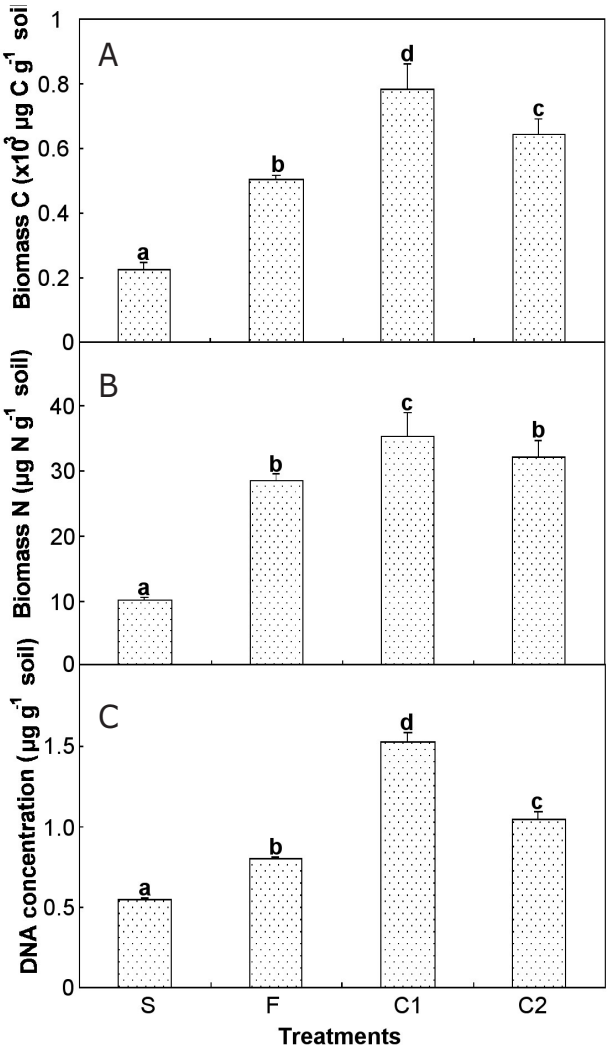


FIG. 1 - Influence of compost rate on the biomass C (A), biomass N (B) and DNA concentration (C). S: soil without amendment; F: farmyard manure at 40 t $ha^{-1}$ treated soil; C1: fompost at 40 t $ha^{-1}$treated soil; C2: fompost at 80 t $ha^{-1}$ treated soil. Means followed by the same letter are not significantly different according to the Newmann and Keuls test at $P < 0.05$; bars are standard deviation.

The microbial N ratio ($B_N$ / Norg) increased in C1-treated soil and F-treated soil and this increase was of 39.7 and 47.6, respectively (Table 2). However, the application of C2 decreased the microbial N ratio wich is of 17.0. The results showed that the high level of compost enriched the soil in organic N instead of microbial N.

**Humic acid and protein impurities in soil and organic treatments**

Soil DNA was often contaminated with humic acid or proteins that interfered with accurate quantification of DNA by UV absorbance at 260 nm (Tebbe and Vahjen, 1993; Kuske *et al*., 1998). The *A260 /A230* and *A260 /A280* ratios for soil DNA were significantly lower than the ratios for DNA solutions from pure cultures (Zhou *et al*., 1996) showing that soil DNA was coextracted with humic compounds (Table 3).

DNA extracts from the C1-treated soil showed higher *A260/A280* and *A260/A230* ratios than those obtained with other treatments. The F-treated soil showed the lowest ratio which may due to the high proportion of humic acids and proteins added to soil after farmyard manure application. Accordingly, the decrease in the microbial biomass C and N and DNA concentration in the C2-treated soil could not be explained by the inhibition effects of proteins or humic acid. So these results may be explained by the presence of other inhibitory source such as the cumulative effect of heavy metals after three years of compost application.

TABLE 3 - Comparison of soil DNA yields and purity

| Treatments | DNA yield (µg DNA $g^{-1}$ dry wt soil) | $A_{260}/A_{280}$ ratio | $A_{260}/A_{230}$ ratio |
|---|---|---|---|
| S | 0.54 (0.06) | 1.23 (0.05)b | 0.84 (0.02)b |
| F | 0.81 (0.05) | 1.05 (0.05)a | 0.71 (0.03)a |
| C1 | 1.52 (0.04) | 1.38 (0.02)c | 0.98 (0.04)c |
| C2 | 1.04 (0.04) | 1.2 (0.03)b | 0.86 (0.03)b |
| Pure culture | | 1.89 | 1.57 |

S: non-treated soil; F: farmyard manure at 40 t $ha^{-1}$ treated soil; C1: compost at 40 t $ha^{-1}$ treated soil; C2: compost at 80 t $ha^{-1}$ treated soil; Pure culture: DNA from Gram positive bacteria.
n = 3 determined by spectrophotometry at 260 nm ($A_{260}$), 280 nm ($A_{280}$) and 230 nm ($A_{230}$); (In brackets): standard deviation; within a column different letter after bracket means that the value is significantly different according to Student-Newmann-Keuls test at $P < 0.05$.

**Heavy metals effect on microbial biomass**

Heavy metals content in soil under different treatments (Table 4) showed an increase in Cd, Ni, Cr, Zn, Cu and Pb with compost addition. The ratio of soil microbial C to soil organic C has been proposed as a useful measure of soil pollution by heavy metals (Brookes, 1995) and a reduction in this ratio as a result of metal has been reported from other studies (Chander and Brookes, 1991; Fliessbach and Reber, 1992). In semiarid conditions, soil biomass is subject to seasonal variations and has an influence on this ratio. Our data showed that the highest rate of MSW compost (80 t $ha^{-1}$) had the lowest ratio of biomass C to soil C (Table 2), indicating a low biomass C content in comparison with the organic C in soil. Similar result was obtained for the ratio of biomass N to soil nitrogen. This low ratio could be attributed not only to heavy metal that had been added with the elevated rates of fertilisers (compost at 80 t $ha^{-1}$), but also to a high condensation and humification of organic matter that is resistant to microbial attack (Tate, 1987). This may account for the results, particularly the low microbial biomass content in the soils amended with MSW compost, compared to the farmyard manure treatment, which is a labile source of organic C for soil biota.

**Relationship between DNA and microbial biomass C and N**

There was a linear relationship between microbial biomass C and microbial biomass N (Fig. 2). Franzluebbers *et al*. (1995) found similar relationship between the microbial biomass C and N. On the other hand, Jedidi *et al*. (2004) found linear relationship in compost treated soil after 2, 4 and 8 weeks of laboratory incubation. A linear relationship between biomass C and DNA concentration was found (Fig. 3B). DNA concentrations and $B_C$ in the soil were highly correlated (Fig. 3B). Nevertheless, the DNA concentration was generally proportional to the $B_C$ and both methods seemed to give reliable values of soil microbial biomass. Similar results were obtained by Marstorp *et al*. (2000), who found a strong relationship between $B_C$, estimated by CFE, and extracted DNA in a mineral soil. They suggested that DNA could be used as a measure of microbial biomass in agricultural soils with low organic matter content. These findings are, however, different from those of Griffiths *et al*. (1997) who found no relationship between $B_C$ and DNA in mineral soils incubated with heavy metals under laboratory conditions. Furthermore, Leckie *et al.* (2004) reported no relationship between DNA yield and $B_C$ in forest humus. The ratios of DNA concentration and $B_C$ and DNA concentration and $B_N$ in the non-treated soil (Fig. 3 A and B) did not differ significantly from the other treated soils.

TABLE 4 - Heavy metals following soil fertilisation

| Treatments | Cd (ppm) | Pb (ppm) | Cr (ppm) | Ni (ppm) | Cu (ppm) | Zn (ppm) |
|---|---|---|---|---|---|---|
| S | 1.11 (0.14)a | 70.46 (13.0)a | 37.58 (4.63)a | 31.88 (3.83)a | 53.14 (2.74)a | 96.97 (3.84)a |
| F | 1.68 (0.21)b | 111.94 (6.24)b | 48.70 (3.58)b | 47.33 (4.07)b | 72.91 (4.51)b | 116.12 (2.19)b |
| C1 | 2.33 (0.21)c | 135.40 (6.61)c | 77.02 (10.11)c | 53.47 (3.16)c | 93.74 (4.57)c | 190.53 (19.01)c |
| C2 | 2.98 (0.35)d | 158.24 (12.11)d | 88.31 (10.69)d | 71.69 (5.76)d | 111.81 (10.23)d | 216.60 (12.65)d |

S: non-treted soil; F: farmyard manure at 40 t $ha^{-1}$ treated soil; C1: compost at 40 t $ha^{-1}$ treated soil; C2: compost at 80 t $ha^{-1}$ treated soil. Cd: Cadmium; Pb: Lead; Cr: Chrome; Ni: Nickel; Cu: Copper; Zn: Zinc.
n = 4; (In brackets): standard deviation; within a column different letter after bracket means that the value is significantly different according to Student-Newmann-Keuls test at $P < 0.05$.

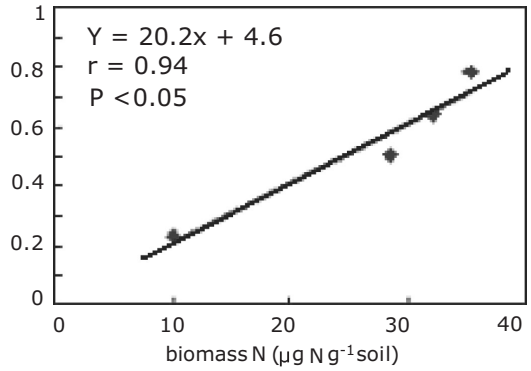


FIG. 2 - Relationship between biomass N and biomass C.

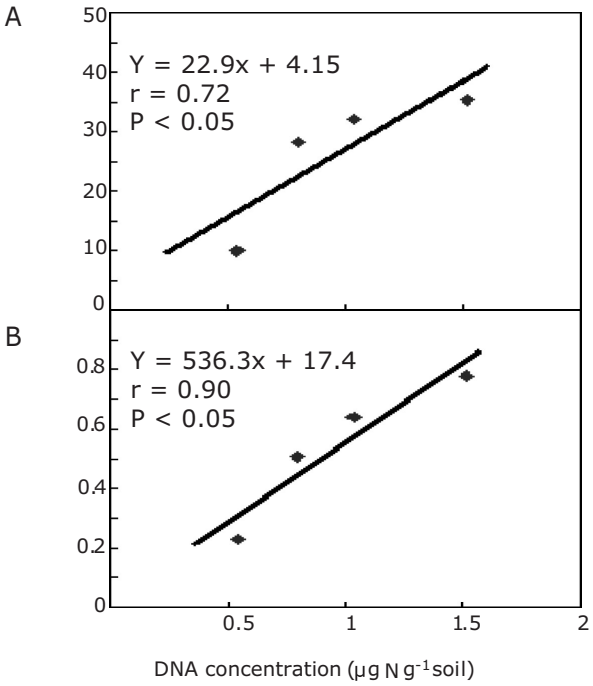


FIG. 3 - Relationship between DNA concentration and biomass N (A) and biomass C (B) in soil.

Moreover the coefficient of variation in the DNA extraction method was lower than the one of the fumigation extraction method (Table 5). These results indicated that the quantification of DNA yields could be used as an alternative and a reliable method than chloroform fumigation extraction method to estimate microbial biomass in cultivated-amended soils.

A correlation matrix (Table 6) shows some significant relationships between the biomass C and N, DNA concentration, micro-organisms counts, total organic C and organic N. There was a strong positive correlation between $B_C$ and $B_N$ or between $B_N$ and DNA concentration. $B_C$ or $B_N$ showed a positive correlation with organic N. However $B_C$, $B_N$ and DNA concentration showed a negative correlation with micro-organisms counts. These results could be explained by the fact that viable and culturable micro-organisms represent only 1 to 10% of the total soil micro-organisms. However the extraction of DNA involved the total soil micro-organisms, including culturable and non culturable ones.

TABLE 5 - Coefficients of variation (%) of microbial C biomass ($B_C$), microbial N biomass ($B_N$) and DNA concentration in treated soil

| Treatments | Coefficients of variation (%) | | |
|---|---|---|---|
| | BC | BN | DNA concentration |
| S | 9.7 | 3.9 | 1.8 |
| F | 14.8 | 12.6 | 6.2 |
| C1 | 11.2 | 7.6 | 2.6 |
| C2 | 6.1 | 4.9 | 2.8 |

S: non-treated soil; F: farmyard manure at 40 t $ha^{-1}$ treated soil; C1: compost at 40 t $ha^{-1}$ treated soil; C2: compost at 80 t $ha^{-1}$ treated soil.

TABLE 6 - Pearson's correlation coefficients between the microbial C biomass ($B_C$), microbial N biomass ($B_N$), DNA concentration, microorganisms counts, TOC and $N_{org}$

| | $B_C$ | $B_N$ | DNA | Microbe counts | TOC | $N_{org}$ |
|---|---|---|---|---|---|---|
| $B_C$ | 1 | 0.91** | 0.92** | −0.41 | 0.44 | 0.52* |
| $B_N$ | | 1 | 0.83** | −0.46 | 0.46 | 0.58* |
| DNA | | | 1 | −0.32 | 0.28 | 0.37 |
| Microbe counts | | | | 1 | −0.23 | −0.31 |
| TOC | | | | | 1 | 0.90** |
| $N_{org}$ | | | | | | 1 |

TOC: total organic carbon; $N_{org}$: organic nitrogen; * correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level.

## CONCLUSION

The application of mature municipal solid waste compost at 40 t $ha^{-1}$ was the best rate which improves soil microbial biomass and DNA extracts in wheat cultivated soil. However the application of municipal solid waste compost at 80 t $ha^{-1}$ enriched the soil on organic C and N. And this rate included a higher content of heavy metals have a negative effect on soil microbial biomass growth. Besides, it exists a significant correlation between microbial biomass C, microbial biomass N and DNA extracts. Moreover the coefficient of variation in the DNA extraction method was lower than the one of the fumigation extraction method. These results indicated that the quantification of DNA yields could be used as an alternative and a reliable method than chloroform fumigation extraction method to estimate microbial biomass in cultivated-amended soils.

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