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# MEASUREMENT OF MICROBIAL BIOMASS AND ACTIVITY IN LANDFILL SOILS

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Two complementary techniques, which have been widely used to provide a general measure of microbial biomass or microbial activity in natural soils, were evaluated for their applicability to soils from the Mallard North and Mallard Lake Landfills, DuPage County, Illinois, U.S.A. Included were: (1) a potassium sulphate extraction technique with quantification of organic carbon for measurement of microbial biomass; and (2) an arginine ammonification technique for microbial activity. Four profiles consisting of replaced soils were sampled for this study; units included topsoil (mixed mollisol A and B horizons), compacted clay cover (local calcareous Wisconsinan age glacial till), and mixed soil/refuse samples. Internally consistent results across the four profiles and good correlations with other independent indicators of microbial activity (moisture, organic matter content, nitrogen, and phosphorus) suggest that, even though these techniques were developed mainly for natural mineral soils, they are also applicable to disturbed landfill soils.

Key Words—Landfill microbiology, landfill soils, arginine ammonification, microbial biomass.

## 1. Background

A variety of techniques are available to provide general and specific measures of microbial biomass or microbial activity in soils. The general techniques include: (1) several for microbial biomass [fumigation–incubation (Jenkinson & Powlson 1976, Shen *et al.* 1984); fumigation–extraction (Brookes *et al.* 1985, Vance *et al.* 1987, Amato & Ladd 1988); and substrate-induced respiration (Anderson & Domasch 1978)]; (2) a technique for microbial volume by microscopy (Jenkinson *et al.* 1976); (3) arginine ammonification for microbial activity (Alef & Kleiner 1986); and (4) measurement of ATP (adenosine 5′-triphosphate) (Jenkinson & Oades 1979, Eiland 1983, Jenkinson 1988). The utility of general techniques must be weighed against more specific techniques which target specific trophic groups. The latter include a wide variety of most probable number (MPN) and plate count techniques. Often, techniques are used in combination for particular soils.

Of the nonspecific techniques, two that have been widely applied to mineral soils (Vance *et al.* 1987, Alef *et al.* 1988) and, to a limited extent, organic soils (Ocio & Brookes 1990, Sparling *et al.* 1990) are: (1) a potassium sulphate extraction technique for determination of microbial biomass carbon (Sparling & West 1988); and (2) an arginine ammonification technique for microbial activity (Alef & Kleiner 1986). Both techniques are relatively quick, require minimal laboratory apparatus, and have been tested on mineral soils with a high degree of correspondence to more cumbersome general and specific techniques. If applicable to disturbed or replaced soils, such

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techniques provide a powerful tool to evaluate microbial dynamics in temporal or spatial dimensions. We report here a preliminary study to examine the applicability of these techniques to disturbed mineral and organic soils within the first 7 m of the surface at a sanitary landfill. The soils include replaced topsoil, replaced silty clay till constituting the landfill cover, and till intercalated with refuse below 2 m.

The overall purpose is to develop techniques to better understand microbial activity in a landfill setting. Many chemical transformations in landfills are mediated by microbial processes—carbon and nitrogen cycling in revegetated cover soils; oxidation of methane by aerobic methanotrophs in cover materials; refuse decomposition by hydrolytic and cellulytic microorganisms; and, ultimately, methane production by methanogenic bacteria. As a result, landfills contain a wide variety of opportunistic microorganisms in complex associations. For better understanding of the spatial and temporal variability of microbial processes in a landfill setting, nonspecific techniques are favoured which include the contribution of as many microorganisms as possible. Ideally, the results of nonspecific techniques should correspond to other indicators of microbial activity in soils, including moisture, organic carbon, nutrient content, and aeration status. In addition, these techniques may have the potential, by modifying the conditions of incubation, to evaluate the activity of particular trophic groups. It should be noted that, because the methanogens are strict anaerobes with specialized metabolic requirements, only non-methanogenic activity is evaluated by the general techniques discussed herein.

## 2. Materials and methods

Samples were taken from the Mallard North and adjacent Mallard Lake Landfills in northern DuPage County, Illinois, U.S.A. Mallard North is a small, 17-ha mounded landfill completed in 1974. At that time, the refuse was covered with 1–2 m of compacted silty clay which was, in turn overlain by 0.3–0.5 m topsoil and seeded with a standard grass mixture. The topsoil and silty clay cover materials were both locally derived. The silty clay cover consists of replaced Wadsworth Till Member of the Wedron Formation, a calcareous late Wisconsinan unit ubiquitous over northeastern Illinois (Willman 1971, Bogner 1988). The topsoil includes the A and B horizons of several soil series of prairie origin (mollisols) formed on top of the Wadsworth itself or a thin loess cap (Mapes 1979). The Mallard Lake site is a 100-ha active landfill with cover materials identical to those at Mallard North. At the time of sampling, the cover had been recently placed at Mallard Lake and no vegetation was yet established. We primarily sampled deeper mixed soil/refuse materials at Mallard Lake to obtain additional deep samples below 2 m.

At Mallard North, two paired profiles (four sample sets in all) were collected in August 1989, near the highest point on the landfill mound about 15 m above the surrounding land surface. A small rotary rig (Giddings) was used to obtain core (0–50 cm) and auger (50–250 cm) samples. Judging by the dense grass cover and the lack of multiple cover/topsoil sequences, the chosen profiles penetrate refuse that was covered and revegetated at the time of landfill closure (1974) without subsequent remedial placement of cover, as has occurred on some portions of the site. Soil samples were preserved in Ziplok bags and refrigerated. At Mallard Lake, deeper samples (1–6 m) were collected in October 1989, on the south face of the south hill approximately 30 m above the surrounding land surface. These were bulk samples (>2 kg each) taken

by hand in the centre portion of large cores obtained with a bucket auger which was used to drill gas recovery wells. Core dimensions were approximately 60 cm (diameter) by 150 cm long. At a depth of 4 m, undecomposed paper yielded a 1983 date. All samples from Mallard Lake consisted of mixed soil and refuse; these were also preserved in Ziplok bags and refrigerated.

Samples were sieved (no.10, 2 mm), thoroughly mixed, and stored at 4°C. As a result of the sieving, large refuse particles were removed from the deepest samples. Background analyses included gravimetric water content, volatile solids (1 h at 550°C), Kjeldahl nitrogen, total phosphorus, and Walkley–Black organic carbon. References for methods are given in Table 1.

The microbial biomass technique (Vance *et al.* 1987) is a simple fumigation–extraction technique in which potassium sulphate is used to extract organic carbon from fumigated and unfumigated soil samples which have been uniformly incubated. Six subsamples of 10 g each are needed for each sample to provide three replicates for fumigated and non-fumigated treatments, respectively. All subsamples were initially placed in an air-tight high humidity chamber for 24 h. For the non-fumigated samples, the extraction consisted of adding 50 ml of 0.5 M potassium sulphate to each subsample, shaking for 30 min (wrist action shaker), centrifuging for 10 min, and filtering the supernatant. A commercial organic carbon analyser (Sybron) was used to determine dissolved organic carbon (DOC) in the supernatant. For the fumigated samples, 15 ml of ethanol-free chloroform was placed in the high humidity chamber for an additional 24 h, after which the chamber was flushed with air for 10 min and the samples extracted and analysed as described above. The extracted organic carbon from the unfumigated sample functions as a control, to be subtracted from the extracted organic carbon of the fumigated sample, which includes dead cell matter. The difference, or "flush" is correlated with microbial biomass and is expressed as mg DOC $g^{-1}$ (dry) sample.

The arginine ammonification technique for microbial activity (Alef & Kleiner 1986, Alef & Kleiner 1987, Alef *et al.* 1988) consists of quantifying the ammonia liberated when a given quantity of arginine is utilized by microorganisms. Parallel incubations under air and nitrogen were used in this study to give relative measures of aerobic and (facultative) anaerobic activity. Twelve 2 g subsamples were required for each sample. All subsamples were incubated at room temperature for 24 h—six under air and six under a nitrogen atmosphere. Then, 0.5 ml of 0.2% (w/v) L-arginine solution was added to all subsamples and six of the subsamples (three anaerobic and three aerobic) stored at 4°C until extracted (Anaerobic arginine was added to the anaerobic samples; this was prepared by bubbling nitrogen through the arginine solution). For the other six subsamples, arginine was similarly added and three were incubated with air and three with nitrogen for 3 h at 30°C, after which they were frozen at 4°C. Ammonia was extracted from all subsamples by adding 8.0 ml of 2.0 M potassium chloride to each sample, vortexing for 15 s, shaking for 15 min (180 rev $min^{-1}$), and centrifuging for 10 min. The supernatant was analysed colorimetrically for ammonia (using absorbance at 630 nm). Ammonia concentrations were converted into an ammonia production rate correlative with microbial activity, as follows:

$$\frac{(3\text{ h ammonia-N}) - (\text{initial ammonia-N})}{(3\text{ h}) \times (\text{dry weight of 1 g wet soil})} = \frac{\mu\text{g ammonia-N}}{\text{g (dry) soil h}^{-1}}$$

TABLE 1
Physical and chemical properties of landfill soils (all values dry weight basis)

| Sample description | Depth (cm) | % Organic C[a] | (SD) | Kjeldahl N[b] (ppm) | (SD) | Total Phosphorus[c] (ppm) | (SD) | % Water[d] | (SD) | % Volatile solids[e] | (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replaced topsoil | 10 | 3.53 | (0.39) | 1710 | (570) | 455 | (56) | 24.27 | (2.84) | 7.65 | (0.90) |
| Mixed topsoil/ | 25 | 2.90 | (1.02) | 1034 | (513) | 396 | (81) | 20.19 | (3.03) | 5.57 | (1.35) |
| Compacted clay | 50 | 2.22 | (0.20) | 844 | (149) | 294 | (38) | 13.05 | (1.00) | 3.16 | (0.25) |
| Cover | | | | | | | | | | | |
| Compacted clay | 100 | 1.55 | (0.34) | 654 | (61) | 269 | (30) | 11.65 | (0.90) | 2.79 | (0.42) |
| Cover | 150 | 1.71 | (0.06) | 545 | (22) | 256 | (17) | 12.45 | (0.80) | 2.36 | (0.37) |
| | 200 | 1.69 | (0.18) | 587 | (50) | 275 | (40) | 12.93 | (0.42) | 2.18 | (0.63) |
| Mixed refuse/ | 250 | 3.13 | (0.73) | 780 | (89) | 296 | (0) | 14.98 | (1.65) | 5.06 | (0.30) |
| Compacted clay | | | | | | | | | | | |
| Cover | | | | | | | | | | | |

[a] Walkley-Black (Sobek *et al.* 1978, Nelson & Sommers 1986).
[b] Technicon (1977), Bremner & Mulvaney (1986).
[c] Technicon (1977), Olsen & Sommers (1986).
[d] Gravimetric moisture content, oven dry overnight at 110°C.
[e] Volatile solids, ignite for 1 h at 550°C.
SD, standard deviation.

## 3. Results and discussion

Selected physical and chemical properties of the landfill soils are shown in Table 1. Three major units are present, as previously discussed: (1) replaced topsoil; (2) compacted calcareous silty clay cover; and (3) mixed refuse/clay at the top of the refuse sequence. In addition, a transitional unit is present between (1) and (2), consisting of mixed topsoil and clay cover. Properties are averaged for the various depths. In a given profile, unit boundaries may be indistinct due to placement of soils by heavy equipment which smeared and mixed contacting units. Nevertheless, contrasts in moisture content, indigenous nutrients (N,P), and measures of organic matter (Walkley–Black organic carbon, VS) can be observed between the replaced topsoil and clay cover, with the topsoil yielding higher values. The mixed clay/refuse is characterized by higher organic carbon content and volatile solids than the clay cover, reflecting the contribution of organic materials in the refuse.

Table 2 gives microbial biomass and microbial activity results for the units described above. For this table, the 1–6 m Mallard Lake samples were averaged with the mixed clay/refuse samples from Mallard North; the combined sample set averaged 16% water and 4% VS. General trends for biomass DOC and aerobic arginine ammonification correspond to the physical and chemical properties shown in Table 1: highest in the topsoil, lowest in the clay cover, and intermediate in the mixed clay/refuse. For the anaerobic arginine ammonification results, results also parallel the physical and chemical properties. Previous studies at Mallard North (Bogner & Moore 1986, Bogner *et al.* 1987, Bogner unpublished data) indicated that measurable oxygen was always present in subatmospheric concentrations at the top of the refuse sequence. Thus, the natural soils would favour both aerobes and facultative anaerobes, with dominance depending largely on seasonal soil moisture dynamics. An infiltration study at Mallard North (Booth & Price 1989) indicated that the topsoil readily transmitted water downward to the topsoil/clay cover interface, which was a zone of preferential horizontal moisture movement. Further downward infiltration was largely confined to widely-spaced vertical fractures in the clay cover. Independent field examination of the vertical fractures indicates that some penetrate the top of refuse with the refuse/clay cover interface a possible secondary zone for horizontal moisture transport. It is suggested, therefore, that the DOC and arginine ammonification results are reasonably representative of *in situ* soils at Mallard North, with higher microbial activity related to higher moisture contents at and above the topsoil/clay cover interface and at the base of the clay cover.

Ratios for specific activity and biomass:carbon are also given in Table 2. The specific activity is a simple measure of the active biomass, calculated from the ratio of the microbial activity to the microbial biomass. In general, these numbers are very low, suggesting that the active portion of the biomass (as measured by the arginine ammonification technique) is very small. Note that this is a comparison of ammonia-N to organic carbon, converted to standard units. As seen in previous trends, higher numbers are characteristic of the surface and deep soils; an exception is the deepest "anaerobic" sample. The biomass: organic carbon is calculated from the ratio of the microbial biomass given in Table 2 to the Walkley–Black organic carbon given in Table 1. Values range from a high of about 8.5 for the surface soil to about 2.2 for the deeper clay cover materials. No obvious increase is noted for soils in the upper refuse sequence (deepest sample). For comparison, surface soils under prairie vegetation in northern Illinois typically have values for the biomass:carbon ratio which range around 2.0 (R.M. Miller unpublished data). Thus, the landfill soils appear to have substantially higher values for this ratio.

TABLE 2
Microbial biomass and microbial activity for landfill soils

| Depth (cm) | Microbial biomass (mg $DOC^{-1}g$ dry soil) | (SD) | Microbial activity ($\mu g\ NH_3-N^{-1}g$ dry $soil^{-1}\ h$) Aerobic | (SD) | Anaerobic | (SD) | Specific activity Ratio Aerobic | Anaerobic | Biomass: C Ratio |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 299.8 | (72.48) | 3.05 | (0.284) | 1.33 | (0.156) | 1.02 | 0.44 | 8.49 |
| 25 | 160.0 | (59.65) | 1.74 | (0.822) | 0.71 | (0.124) | 1.09 | 0.44 | 5.52 |
| 50 | 62.88 | (25.89) | 0.35 | (0.608) | 0.06 | (0.339) | 0.56 | 0.10 | 2.83 |
| 100 | 53.76 | (14.63) | 0.49 | (0.193) | 0.05 | (0.087) | 0.91 | 0.09 | 3.47 |
| 150 | 37.39 | (18.62) | 0.27 | (0.185) | 0.15 | (0.113) | 0.72 | 0.40 | 2.18 |
| 200 | 55.96 | (32.35) | 0.38 | (0.319) | 0.17 | (0.246) | 0.68 | 0.30 | 3.31 |
| 250–600 | 104.3 | (40.56) | 1.76 | (1.123) | 0.14 | (0.271) | 1.69 | 0.13 | 3.33 |

DOC, dissolved organic carbon; SD, standard deviation.

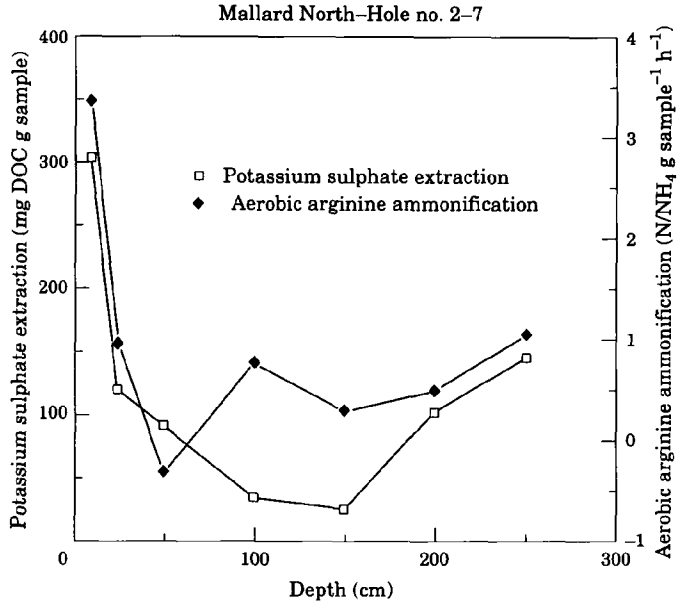


Fig. 1. Profile for microbial biomass and aerobic arginine ammonification at Mallard North, Hole 2–7.

The typical shape of profiles for microbial biomass and microbial activity is shown in Fig. 1. This profile compares results for microbial biomass and aerobic microbial activity for Hole 2–7 at Mallard North. The other three profiles at Mallard North show similar trends for these variables, with high surface values which decline with depth but rise again in the top of refuse. For purposes of data analysis, results from the four adjacent profiles were averaged. Standard deviations were given previously in Table 2.

Microbial biomass and activity were, in general, highly correlated with each other and with other variables that might be positively correlated with microorganism activity in these soils. Figure 2 presents simple linear regressions for the microbial biomass and microbial activity results when intercomparisons are made among the three data sets. The $R^2$ values for the linear regression, which is the square of the linear correlation coefficient and can be used as a rough measure of the fraction of variation of one variable which is described by the paired variable, range from 0.78–0.94. The low value for $R^2$ in Fig. 2c (for the comparison of anaerobic to aerobic activity) may be partially explained by the very low values for anaerobic activity and might be improved with a more controlled anaerobic method. Figure 3 illustrates similar linear regressions between microbial biomass and % organic carbon, % water, % volatile solids, Kjeldahl N, and total P. The $R^2$ values range from 0.73–0.96. For comparison, the $R^2$ values for linear regressions with the same variables (organic carbon, water, volatile solids, Kjeldahl N, total P) to aerobic microbial activity range from 0.81–0.97 and for anaerobic microbial activity from 0.56–0.95. The lowest value (0.56) was again associated with a correlation for anaerobic activity, in this case to organic carbon.

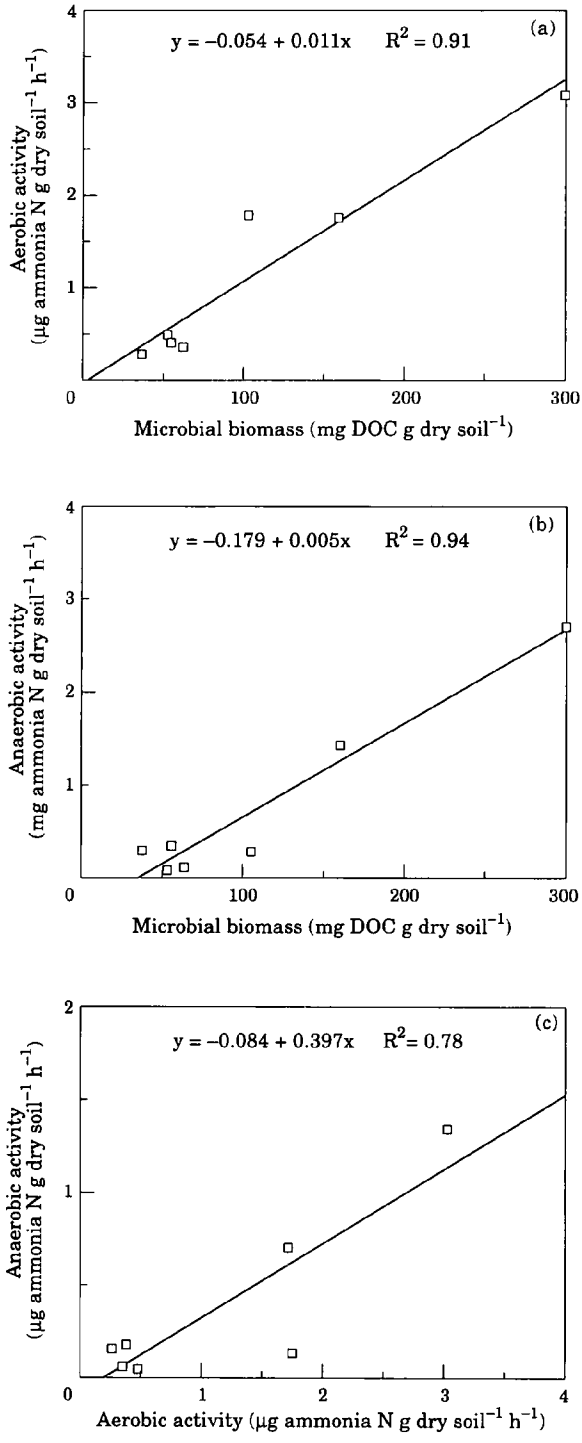


Fig. 2. Linear regressions between microbial biomass and microbial activity. (a) microbial biomass to aerobic activity; (b) microbial biomass to anaerobic activity; (c) aerobic activity to anaerobic activity.

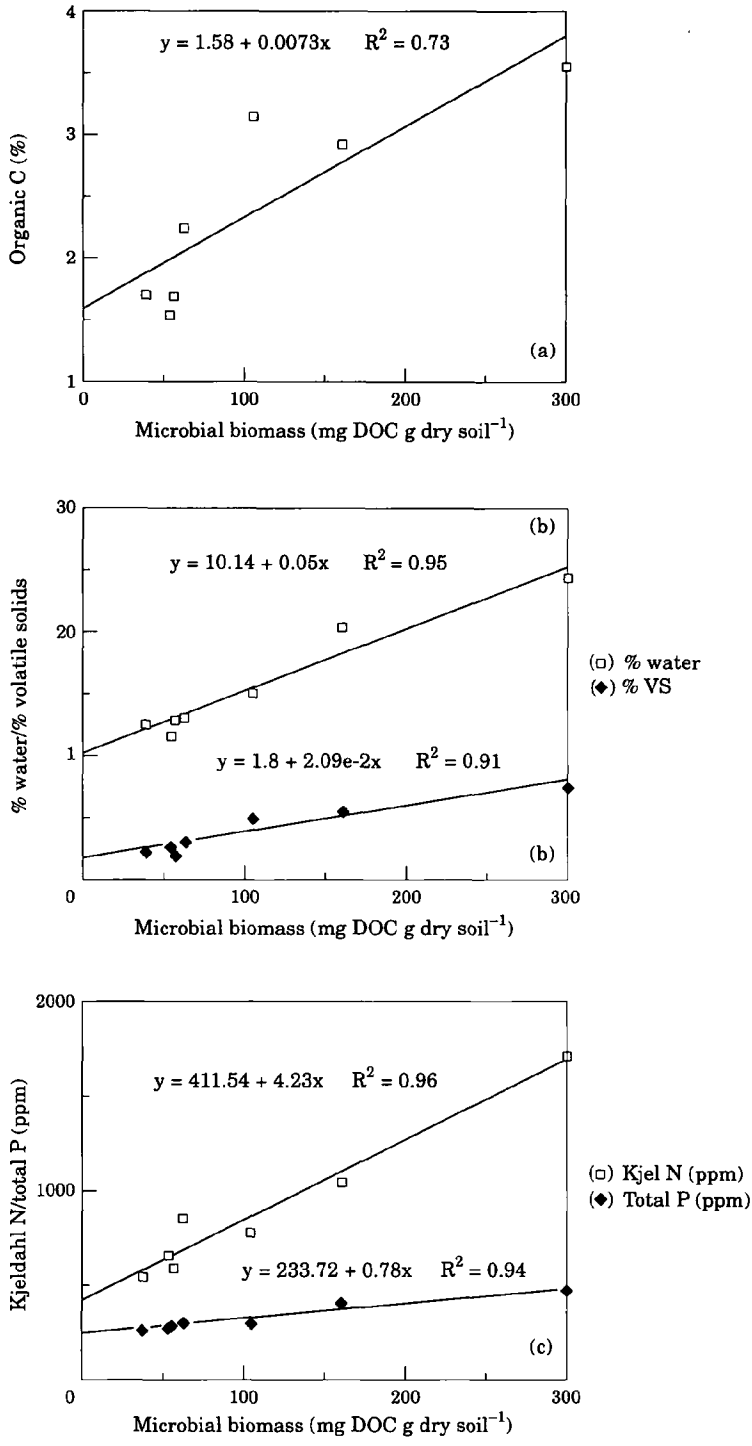


Fig. 3. Linear regressions between microbial biomass and selected other variables. (a) microbial biomass to organic carbon content; (b) microbial biomass to water content, volatile solid content; (c) microbial biomass to Kjeldahl N, total P.

## 4. Conclusions

This preliminary study has indicated the utility of the potassium sulphate extraction and arginine ammonication techniques for landfill soils. Results for the Mallard profiles were internally consistent and highly correlated to organic carbon, water content, volatile solids, and major nutrients (N,P). Further verification of the applicability of these techniques to a range of other landfill soils with variable physical and chemical properties is needed. In addition, investigations using altered conditions of incubation to target specific trophic groups, for comparison with more direct enumeration techniques, is strongly recommended. As a minimum, microbial biomass and microbial activity would appear to be robust indicators of reclamation success at landfills, as they indicate the presence or absence of a flourishing soil microbial community that complements revegetation efforts. More generally, the results to date suggest that these techniques may be useful indicators of both spatial and temporal variability in microorganism activity at landfills and in other disturbed soils.

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