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GREENS MANAGEMENT

Accumulation of Microbial Biomass within Particulate Organic Matter of Aging Golf Greens

Mine Kerek, Rhae A. Drijber,* William L. Powers, Robert C. Shearman, Roch E. Gaussoin, and Anne Marie Streich

ABSTRACT

Microbial biomass (MB) is a key variable controlling soil organic matter dynamics in soil. Currently, there is little information on the amount and significance of MB in highly managed golf greens. Our objective was to determine the amount and distribution of MB within soil structural components of golf greens and its relationship to the location of organic substrates. During 1996, 47 greens were sampled from 12 golf courses within Nebraska (USA). Microbial biomass, determined as extractable lipid phosphate on field-moist soils, increased linearly with age of green ($Y = 19.39 + 3.54x$; $r^2 = 0.87$, $P = 0.001$). In 1997 and 1999, selected greens were resampled and separated into mineral fraction (MF) and particulate organic matter (POM) fraction using a sodium metatungstate (NMT; $r = 2.3 \text{ g cm}^{-3}$). Then, POM was separated into light (L-POM) and heavy (H-POM) fractions using NMT ($r = 2.0 \text{ g cm}^{-3}$). Amount of MB of whole soil and POM was linearly related to green age ($r^2 = 0.76$ and 0.68 , respectively). Amount of MB in MF was not related to green age. The portion of total soil MB associated with POM increased significantly from 25.6% for an 8-yr-old green to 77.8% for a 28-yr-old green. Carbon in fulvic acid and humic acid increased with green age from 0.5 to 1.7 and 0.6 to 2.6 g kg^{-1} soil, respectively. As humus is a relatively stable form of soil organic matter, we hypothesized that humus accumulation within POM renders both POM and associated MB more resistant to degradation; thus, they accumulate.

GOLF GREENS ARE TURF SURFACES constructed of very sandy soil mixtures. Soil processes important for maintaining healthy turfgrass, such as organic matter degradation, nutrient supply, N fixation, mycorrhizal colonization, and disease occurrence and suppression, are mediated by the soil microbial biomass (MB) (Alexander, 1977; Nelson, 1992, 1994; Gregorich et al., 1994; Turgeon, 1996). Microbial biomass also serves as a labile source of organic matter (Gregorich et al., 1994) and as a source and sink for the major plant nutrients. Therefore, MB is essential to turfgrass health and long-term green productivity (Nelson, 1994). Despite its known importance, there is little information on the amount and significance of MB in golf greens. Populations of total fungi and total bacteria, as well as selected microbial groups, have been enumerated in golf greens by plating onto various media (Mancino et al., 1993; Liu et al., 1995; Elliott and Des Jardin, 1999a, 1999b). A bias is instantly introduced into the analysis when using

a culturable method as one obtains only those organisms that can be cultured on a particular medium under defined conditions (Elliott and Des Jardin, 1999b). Therefore, cultural techniques significantly underestimate the MB of golf greens. An alternative method of determining MB is based on extraction of cellular components (e.g., phospholipids) from cells within the soil (Frostegård et al., 1991). This method does not rely on recovery of intact, viable, and culturable cells but requires quantitative extraction of cellular components from cells within the soil and selection of appropriate conversion factors to calculate MB (Hill et al., 1993). Although this approach has not been applied to golf greens, it has been used successfully to quantify MB in other ecosystems (Findlay et al., 1989; Frostegård et al., 1991; Hill et al., 1993; Jordan et al., 1995; Drijber et al., 2000).

Microbial biomass and its products may be associated with free primary soil particles (i.e., sand, silt, and clay), aggregates, and macroorganic matter (Tisdall and Oades, 1982; Ahmed and Oades, 1984; Kanazawa and Filip, 1986; Beare et al., 1990; Gregorich and Janzen, 1996). The location of microorganisms in the soil structure has been studied by different methods, such as electron microscopy (Foster, 1988), repeated washing of soil aggregates (Hattori, 1988), or by methods involving physical soil fractionation and density separation (Turchenek and Oades, 1979; Monrozier et al., 1991; Christensen, 1992). Factors determining the distribution of microorganisms in the soil structure, such as substrate location, clay content, microaggregation, and resistance to drying, have been used to explain microbial survival in soils (Van Gestel et al., 1996). In highly sandy soils, such as golf greens, mechanisms promoting the development, survival, and beneficial activities of MB are largely unknown. Thus, our objective was to determine the amount and distribution of MB within soil structural components of golf greens and its relationship to the location of organic substrates.

MATERIALS AND METHODS

Soil Samples

The study consisted of 47 golf greens sampled during the fall of 1996 from 12 golf courses located within Nebraska (USA). Of the 47 greens, six selected greens were resampled

Abbreviations: CC, country club; FA-C, fulvic acid carbon; HA-C, humic acid carbon; H-POM, heavy particulate organic matter; lipid-P, extractable lipid phosphate; L-POM, light particulate organic matter; MB, microbial biomass; MF, mineral fraction; NMT, sodium metatungstate; POM, particulate organic matter.

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from five courses in the fall of 1997, and an additional six greens were resampled from three courses in the spring of 1999. Soils from 10 or more cores (2 cm in diam. by 15 cm in depth) were composited from each green after removal of grass thatch. Soils were kept on ice for transport to the lab where they were refrigerated for less than a week before the removal of visible roots and sieving to 4 mm. During sieving, numerous sand particles were attached to the roots, and thus stayed on the sieve. In order to include these particles in the soil mixture, material staying on the sieve was air-dried for a few hours while refrigerating material passing through the sieve. After a second sieving, excluding roots as much as possible, particles passing through the sieve were mixed with previously sieved soil material. After mixing to homogenize, the sample was divided into two parts. One part was subsampled immediately for MB determination, with the remaining soil frozen field-moist at -22°C . The second part was air-dried and sieved to 2 mm for further physical and chemical analyses, including MB determination on soil density fractions.

Basic soil characterization was done by the University of Nebraska Soil and Plant Analytical Laboratory. Particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986), total soil C and N were determined by dry combustion using LECO FP-2000 C and N analyzer (LECO Corp., St. Joseph, MI), and pH was determined in a 1:1 soil/water mixture (Thomas, 1996).

Subsampling Procedure for Soil Density Fractionation

Air-dried soil <2 mm was rolled back and forth on a large piece of paper in all directions and then gently spread out in a flat circle of about 1-cm thickness. Both soil particle size and amount of plant material seemed to increase from inner- to outermost part of the circle. Thus, to be representative, a complete wedge-shaped subsample large enough to provide the required amount of soil was removed from the circle. In other words, for different amounts of soil samples needed,

only the curvature length (not the radius) of the wedge was changed. Duplicates were subsampled in the same manner from the remaining soil in the circle.

Separation of Particulate Organic Matter

By definition, particulate organic matter (POM) is separated from the soil mineral fraction (MF) by dispersion in hexametaphosphate and sieving to $53\ \mu\text{m}$ (Cambardella and Elliott, 1992). Material remaining on the sieve, corrected for sand, is called POM while material passing through the sieve is termed MF. Density fractionation often precedes dispersion and sieving to isolate free POM, i.e., that occurring between aggregates (Golchin et al., 1995; Cambardella and Elliott, 1993; Jastrow et al., 1996). Because our objective was to separate soil fractions without redistribution of MB, the soil was not dispersed in hexametaphosphate. Furthermore, because few soil particles from these highly sandy greens would pass a $53\text{-}\mu\text{m}$ sieve, density separation alone was used to separate POM from MF (Fig. 1). Soil samples were taken in duplicate for MB determinations. However, because we obtained very little variation between the replicates for humus C determination, only a few samples were duplicated for this analysis. Air-dried soil samples <2 mm (25 g for humus C and 10 g for MB determination) were placed in a 250-mL centrifuge bottle to which 150 mL of sodium metatungstate (NMT) solution ($3\text{Na}_2\text{WO}_4 \cdot 9\text{WO}_3$, Aldrich Chem. Co., St. Louis) with a density of $2.3\ \text{g cm}^{-3}$ was added (Shaymukhametov et al., 1985). In preliminary experiments, we tested the feasibility of densities of 2.0, 2.2, and $2.3\ \text{g cm}^{-3}$ for POM separation. Separation with a density of $2.0\ \text{g cm}^{-3}$ left visually large amounts of POM within the MF. Another experiment on three selected greens from different age groups compared the densities of 2.2 and $2.3\ \text{g cm}^{-3}$. The result (not shown) was that ash content of the POM separated by a density of $2.3\ \text{g cm}^{-3}$ was even smaller than that of $2.2\ \text{g cm}^{-3}$ in two of the greens. This suggested that the difference in the results due to the two

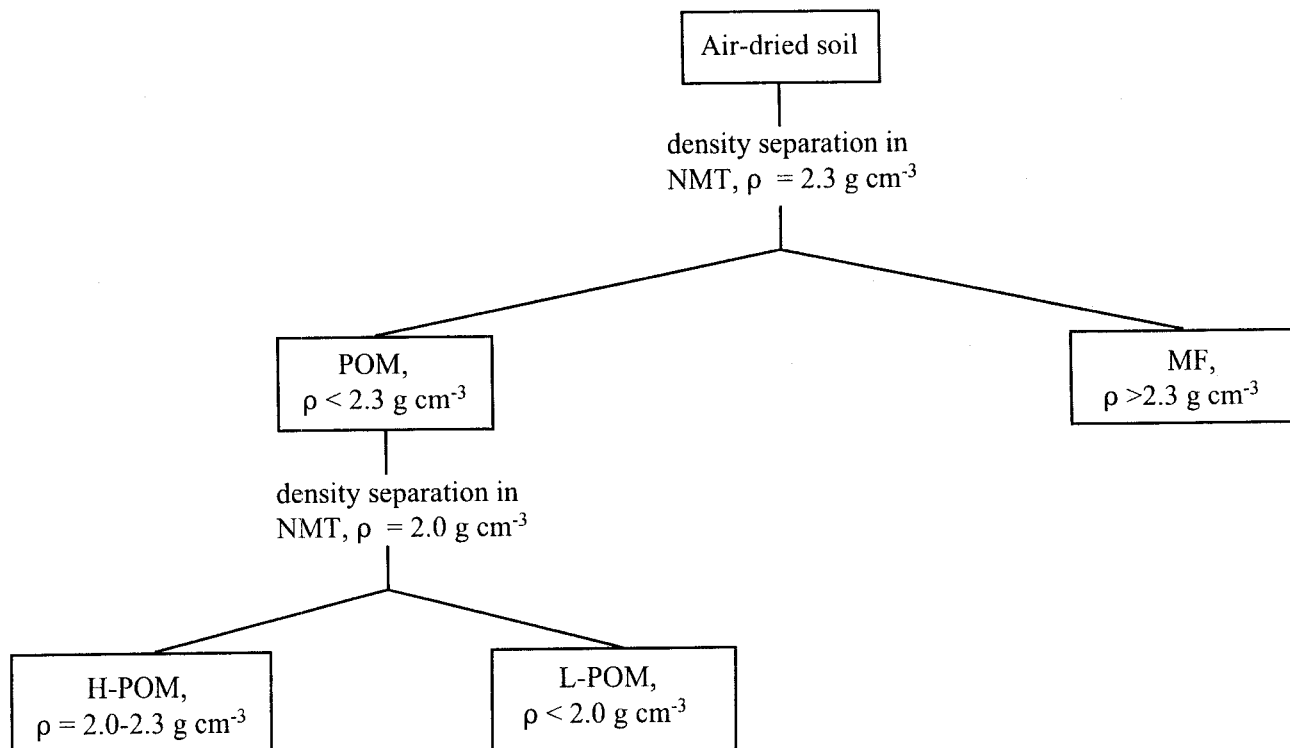


Fig. 1. Soil fractionation sequence for determining the location of microbial biomass (MB). NMT, sodium metatungstate; POM, particulate organic matter; MF, mineral fraction; H-POM, heavy POM; and L-POM, light POM.

Table 1. Physical and chemical characteristics of soils sampled in 1996, 1997, and 1999.†

Golf course	Green no.	Age‡	Sand	Silt	Clay	pH	Age§	Total N	Total C	FA-C¶	HA-C¶	L-POM#	H-POM††	POM‡‡
		yr	— g kg ⁻¹ soil —				yr	— g kg ⁻¹ soil —		— g C kg ⁻¹ soil —		— g kg ⁻¹ soil —		
Country Club (CC) of Lincoln	2	3	910	10	80	6.6	4	0.38	5.7	0.5	0.6	4.8	6.4	11.2
Shadow Ridge CC	7	4	945	25	30	6.5	5	0.32	6.7	0.8	0.9	6.7	7.2	13.9
North Forty	8	7	938	20	42	6.7	8	0.43	7.1	1.0	1.2	6.3	11.8	18.1
Firethorn CC	12	12	943	15	42	6.5	15	0.79	9.6	1.5	1.9	8.6	13.4	22.0
	13	12	923	30	47	6.4	15	0.75	9.3	1.5	1.8	8.4	15.8	24.2
Pines CC (Valley)	10	18	891	45	64	6.6	21	0.82	8.6	1.0	1.2	10.0	11.1	21.1
	18	18	902	45	53	6.5	21	0.84	8.4	1.3	1.4	10.3	14.2	24.5
Mahoney	10	20	913	35	52	6.5	21	1.08	12.5	1.7	2.6	18.4	35.0	53.4
Grand Island Municipal	3	20	922	40	38	6.6	21	1.10	12.1	1.4	1.7	25.6	15.7	41.3
	13	20	914	40	46	6.6	21	1.09	11.8	1.6	1.9	21.3	13.1	34.4
Bayhills	5	25	934	25	41	6.5	28	1.14	10.8	1.5	2.0	22.8	28.4	51.2
	9	25	914	40	46	6.7	28	1.19	11.1	1.4	1.9	21.6	27.7	49.3

† Textural class and pH were determined in soils sampled in 1996. The remaining soil characteristics were determined in soils sampled in 1997 and 1999.

‡ Ages of greens sampled in 1996.

§ Ages of greens sampled in 1997 and 1999.

¶ Sum of C in fulvic acid (FA-C) and humic acid (HA-C) fractions of mineral fraction (MF) and particulate organic matter (POM).

L-POM, light particulate organic matter.

†† H-POM, heavy particulate organic matter.

‡‡ Sum of the masses of L-POM and H-POM.

densities used was small enough to be masked by the variability between any of the two soil subsamples. Therefore, we chose the density of 2.3 g cm⁻³ to be the optimum density for separation of POM from the MF. This separation yielded a POM fraction with some free sand particles, few aggregates, and a MF with the least amount of POM. The bottles were mixed mechanically by hand for about 1 min. The particles that adhered to the lid and bottle were washed into suspension using NMT solution, and the suspension was allowed to stand for 30 min before centrifuging at 2000 g for 30 min (Golchin et al., 1994). The supernatant with floating particles (POM) was recovered by aspiration (Strickland and Sollins, 1987) and then poured into a Millipore filter funnel fitted with a glass-fiber filter paper (Whatman GF/A) and filtered under vacuum. The material remaining in the bottle was designated MF. Total POM and MF were quantitatively washed into appropriately sized centrifuge tubes for MB and humus C determinations.

Separation of total POM, after briefly air-drying, into light POM (L-POM) and heavy POM (H-POM) was achieved as outlined above by density separation in NMT ($\rho = 2.0$ g cm⁻³). Preliminary experiments determined that a density of 2.0 g cm⁻³ yielded the fraction with the highest proportions of undecomposed plant material although it may have included some partially decomposed plant material as well. The POM fractions were quantitatively washed into appropriately sized centrifuge tubes and extracted immediately for MB; the residue remaining after MB extraction was then thoroughly rinsed with distilled water to remove residual NMT before mass balance determination. Total POM, L-POM, and H-POM masses reported in Table 1 include associated mineral particles remaining after fractionation. Mass of total POM was determined as the sum of L-POM and H-POM. Mineral fraction, L-POM, and H-POM ash contents of selected samples were determined by keeping samples at 550°C for 14 h and then at 750°C for 2 h.

Humus Extraction and Fractionation

Humus was extracted from MF and total POM with a combined solution of 0.1 M sodium hydroxide (NaOH) and 0.1 M sodium pyrophosphate (Na₂P₂O₇) and fractionated into humic acid and fulvic acid fractions at pH 1.5 (Lowe, 1980). Carbon in the extracts was determined by the Walkley-Black wet oxidation method (Allison, 1965). Before organic matter extraction, centrifuge bottles containing MF and total POM were placed in an oven at 60°C for several hours to remove the water held by these fractions.

Microbial Biomass Determination

Total POM, MF, L-POM, and H-POM fractions were quantitatively washed into Teflon tubes, and excess water was decanted. Microbial biomass in the above fractions and from field-moist soil was determined as extractable lipid phosphate (lipid-P) using a modified Bligh and Dyer procedure as described in Kates (1986). Briefly, field-moist soils (1–2 g) and wet samples of the above fractions were extracted with 4 mL of a single-phase mixture of methanol (CH₃OH) and chloroform (CHCl₃) in a ratio of 2:1 (v/v) and then with 4 mL of methanol, chloroform, and water in a ratio of 2:1:0.8 (v/v). To separate the phases, 2.5 mL of chloroform and ammonium sulfate [(NH₄)₂SO₄] was added to the combined extracts. For soil fractions of different mass, the volumes were adjusted proportionally. The lower chloroform layer was removed and evaporated under N₂ to dryness. Phosphate released through perchloric acid digestion was determined by the method of Barlett as described in Kates (1986).

Statistical Analysis

Linear regression analysis was performed using PC SAS version 6.12 (SAS Inst., Cary, NC). The purpose of the linear regression was to test the model $Y = \beta_0 + \beta_1 X$, where X is the age of green and Y is the soil property, and determine whether the slope was significantly different from zero.

RESULTS AND DISCUSSION

Soil characteristics of the selected greens sampled in 1996, 1997, and 1999 are given in Table 1. The textural class for all of the greens was sand.

Microbial Biomass of Whole Soil

Analysis of 47 golf greens sampled in 1996 found a significant positive linear relationship of MB (determined as lipid-P on field-moist soils) to green age ($Y = 19.39 + 3.54x$, $r^2 = 0.87$, $P = 0.001$; Fig. 2A). Contributions of plant lipids to lipid-P were minimal based on the absence of the plant fatty acid, linolenic acid, in lipid extracts from the same soils (data not shown). Furthermore, total bacterial fatty acids were strongly correlated with both lipid-P ($r^2 = 0.93$) and green age

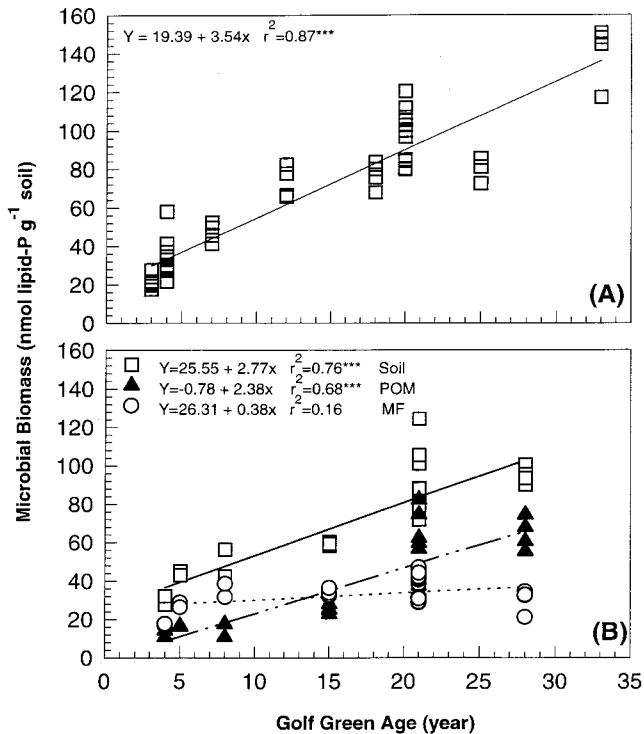


Fig. 2. Microbial biomass (MB) measured as extractable lipid phosphate (lipid-P) on (A) field-moist soils sampled in 1996 and (B) air-dried soils sampled in 1997 and 1999. Microbial biomass on air-dried soils includes those of whole soil (as the sum of MB in POM and MF), particulate organic matter (POM), and mineral fraction (MF). Each data point is a single observation. ***Both model and slope are significant at $P = 0.001$.

($r^2 = 0.80$), supporting lipid-P as an accurate measure of MB in these greens (paper in preparation).

The above relationship on field-moist soils compared favorably with the smaller data set on air-dried soils from 12 golf greens ($Y = 25.55 + 2.77x$, $r^2 = 0.76$, $P = 0.001$; Fig. 2B). Thus, analysis of air-dried soil fractions accurately reflected MB measured on field-moist soils, and exposure to NMT did not interfere with the lipid extraction. The former is in disagreement with the results of West et al. (1988) and Van Gestel et al. (1991) where the largest biomass C decline upon air-drying was found for a sandy soil.

Microbial biomass in soil increased significantly with age of green from a low of 27.7 nmol lipid-P g^{-1} soil for a 4-yr-old green at the Country Club (CC) of Lincoln to a high of 124.5 nmol lipid-P g^{-1} soil for a 21-yr-old one at Mahoney (Fig. 2B). Soil mixes commonly used to construct golf greens contain <15 nmol lipid-P g^{-1} soil, which is derived primarily from the peat in the mix (data not shown). The above range in lipid-P equates to a MB between 249 and 1119 mg C kg^{-1} soil based on conversion factors of 50 $\mu\text{mol P } g^{-1}$ dry cell (White et al., 1979) and 0.45 g C g^{-1} dry cell (Paul and Clark, 1989). For comparison, a silt loam soil in central Iowa cropped to corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] or in virgin tallgrass prairie contained an average biomass, by chloroform fumigation extraction, of 354 and 1145 mg C kg^{-1} soil, respectively (DeLuca and Keeney, 1994). Inclusion of MB from the thatch layer would further support golf greens as a reservoir

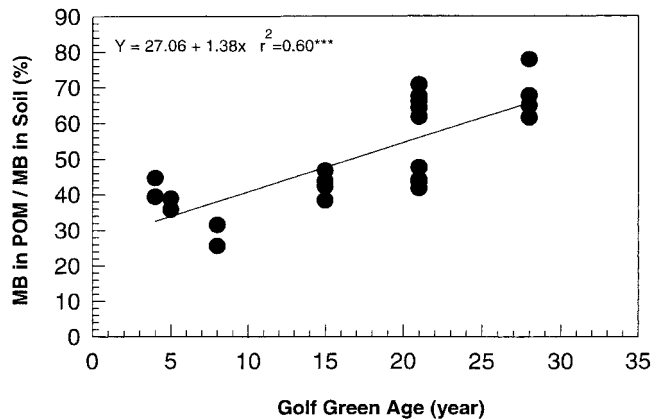


Fig. 3. Microbial biomass (MB) accounted for by particulate organic matter (POM) of air-dried soils from greens sampled in 1997 and 1999. Each data point is a single observation. ***Both model and slope are significant at $P = 0.001$.

of MB as thatch contains an equal or greater number of microorganisms than the mineral soil beneath (Mancino et al., 1993). Preliminary experiments on the above 47 golf greens found that very little of this biomass was associated with water-stable aggregates, which accounted for $<12\%$ of the soil mass (data not shown). Thus, current models for physical protection of MB within aggregates (Hattori, 1973; Ahmed and Oades, 1984; Tisdall and Oades, 1982) could not account for the accumulation of MB within golf greens.

Microbial Biomass of Mineral Fraction and Particulate Organic Matter

Microbial biomass tends to congregate near suitable food sources such as decaying plant and animal debris (Haynes and Beare, 1996). As a result, MB may associate with POM. Kanazawa and Filip (1986) found that 66.6% of total MB, determined by adenosine triphosphate (ATP), was within the light fraction ($\rho < 1 \text{ g cm}^{-3}$) of a loamy sand soil despite the minor contribution of this fraction to the dry soil mass. In addition, Hissett and Gray (1976) showed that 64% of bacteria in a sandy soil were associated with organic particles. On the other hand, MB could associate with mineral soil particles due to their physiological requirements through binding to thin organic coatings on mineral surfaces (Hissett and Gray, 1976) or because of inhibitory substances within POM. Ahmed and Oades (1984), for example, found insignificant amounts of MB associated with the light fraction ($\rho < 1.6 \text{ g cm}^{-3}$) of a fine sandy loam and a clay soil.

To determine the degree of association of MB with mineral soil particles and plant residues, we separated the soils into two density fractions: MF ($\rho > 2.3 \text{ g cm}^{-3}$) and POM ($\rho < 2.3 \text{ g cm}^{-3}$). Ash content of MF was $>98\%$ while POM fractions contained 70 to 78% ash, indicating satisfactory separation of POM from MF despite the high density used for separation. Density fraction procedures are often criticized for their shortcomings in successfully separating the mineral-free plant material from more complex soil organic matter (Meijboom et al., 1995) as ash contents of light fractions mostly exceed 50% (Christensen, 1992).

Microbial biomass in POM increased significantly,

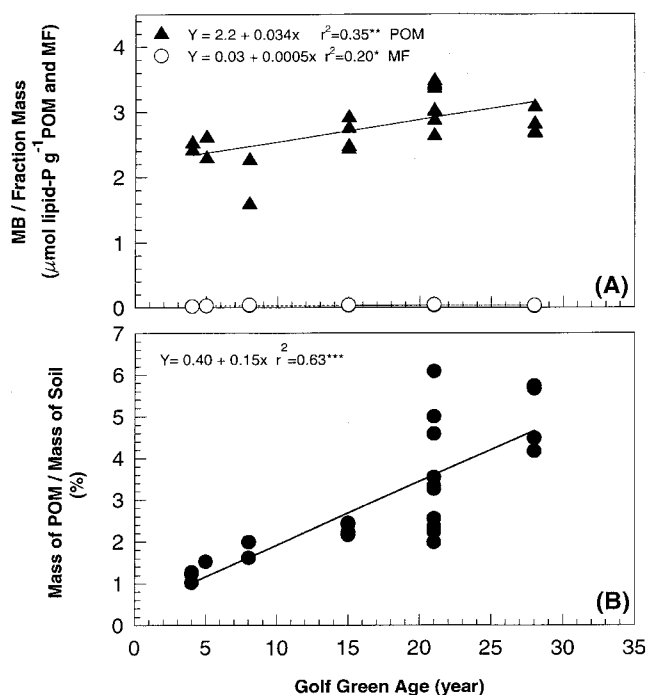


Fig. 4. Relationship to green age of (A) microbial biomass (MB) per unit mass of particulate organic matter (POM) and mineral fraction (MF) and (B) soil mass composed of POM. Soils are air-dried and from greens sampled in 1997 and 1999. Each data point is a single observation. *, **, and ***, both model and slope are significant at $P = 0.05$, 0.01 , and 0.001 , respectively.

whereas MB in the MF remained constant with age (Fig. 2B). This resulted in a significant increase with green age in the portion of total MB included in POM, from 25.6% for an 8-yr-old green to 77.8% for a 28-yr-old one (Fig. 3). In younger greens (≤ 15 yr), the MF comprised a large portion of MB (from 53.2–74.4%), whereas in older ones (> 15 yr), POM comprised a large portion (from 61.5–77.8%). An exception to this occurred in 21-yr-old greens from Pines CC where MF contained 52.3 to 58.2% of total MB.

The increase in the portion of total MB in POM with age of green could result from (i) increased MB per unit mass of POM, (ii) increased mass of POM within a unit mass of soil, or (iii) a combination of the two. Microbial biomass per unit mass of POM and MF is calculated and plotted in Fig. 4A. Although significant ($r^2 = 0.20$, $P = 0.05$), MB per unit mass of MF increased slowly with green age at the rate of $0.45 \text{ nmol lipid-P g}^{-1} \text{ yr}^{-1}$. In contrast, MB per unit mass of POM increased significantly ($r^2 = 0.35$, $P = 0.01$) with green age at the rate of $34.18 \text{ nmol lipid-P g}^{-1} \text{ yr}^{-1}$. Because the above relationships were not strong, we concluded that the increase in the portion of total MB in POM with age resulted mainly from an increase in the soil mass comprised of POM ($r^2 = 0.63$, $P = 0.001$; Fig. 4B).

Microbial Biomass of Particulate Organic Matter Fractions

As the POM was not mineral-free and included few aggregates, our next purpose was to determine whether the amount of MB within POM was related to the degree of association of POM with mineral material. Thus, we

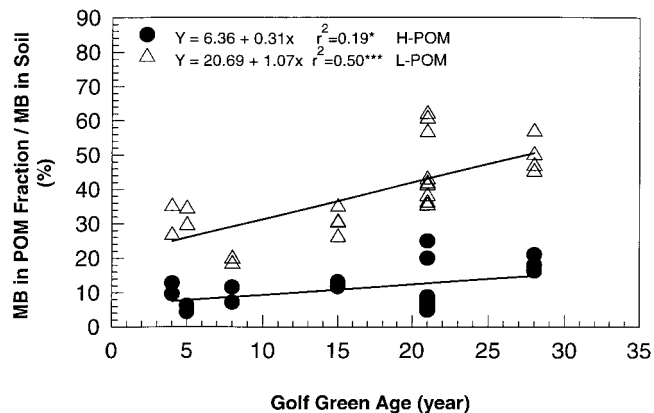


Fig. 5. Microbial biomass (MB) accounted for by heavy particulate organic matter (H-POM) and light POM (L-POM) of air-dried soils from greens sampled in 1997 and 1999. Each data point is a single observation. * and ***, both model and slope are significant at $P = 0.05$ and 0.001 , respectively.

separated the POM into L-POM and H-POM. Microscopic examinations (not presented) showed that L-POM included mostly large, undecomposed root and plant fragments with little mineral material, whereas H-POM contained few small aggregates and free sand particles. The L-POM and H-POM comprised up to 25.6 and 35.0 g kg^{-1} soil, respectively (Table 1). The range in ash content of L- and H-POM was 37 to 58% and 86 to 93%, respectively. Meijboom et al. (1995) found that ash content increased with fraction density, suggesting greater decomposition and/or humification in higher-density fractions (Gregorich et al., 1994). This supports microscopic observations that H-POM is more associated with minerals and appears more altered than L-POM.

Microbial biomass accounted for by L-POM and H-POM increased significantly with age from 18.3 to 61.9% and from 4.4 to 25.0%, respectively (Fig. 5). However, MB was 1.6 to 8.9 times greater in L-POM than in H-POM, indicating preferential accumulation of MB in L-POM. This agreed with the findings of Beare et al. (1990) where plant residues contained a larger and more physiologically active biomass than mineral soil. Thus, L-POM was responsible, in a large part, for the increased MB in total POM.

Humus and its Relationship to Microbial Biomass

Carbon in fulvic acid (FA-C) and humic acid (HA-C) of whole soil (determined as the sum of C in POM and MF) increased with green age from 0.5 to 1.7 g kg^{-1} soil and from 0.6 to 2.6 g kg^{-1} soil, respectively (Table 1). The total soil C accounted for by FA-C and HA-C in MF decreased from a high of 21.3% for a 15-yr-old green at Firethorn CC to a low of 4.8% for a 21-yr-old green at Grand Island Municipal. However, this relationship was not significant (Fig. 6). The total soil C accounted for by FA-C and HA-C in POM significantly increased with age from 10.5% for a 4-yr-old green at the CC of Lincoln to 26.4% for a 21-yr-old green at Grand Island Municipal (Fig. 6).

Our observations led us to speculate why POM, particularly L-POM, which contained less altered plant residues, was accumulating despite associated biomass.

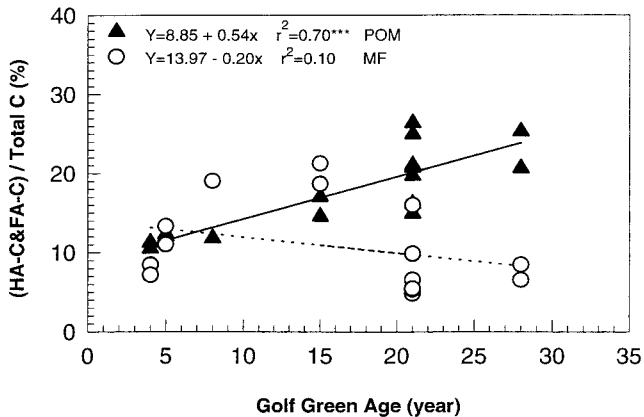


Fig. 6. Total C comprised of C in humic acid (HA-C) and fulvic acid (FA-C) fractions of air-dried soils from greens sampled in 1997 and 1999. Each data point is a single observation. POM, particulate organic matter; MF, mineral fraction. ***Both model and slope are significant at $P = 0.001$.

Some of this accumulation may result from a lag that occurs between the time of organic material deposition and the time of consumption (Elliott et al., 1996). We hypothesized that the factor(s) causing POM to accumulate could also cause associated MB to accumulate. The mechanisms rendering POM more resistant to degradation could relate to the location of soil humus because humus is a relatively stable form of SOM. It is well known that soil microorganisms are essential to humus formation. Therefore, it is conceivable, provided the movement of humic substances is negligible, that the major site of humus accumulation could be related to the predominant microsites for microbial activity in soil. This relationship is supported by Fig. 7 where the amount of humic substances in POM relative to MF increased significantly ($r^2 = 0.92$, $P = 0.001$) with the amount of MB in POM relative to MF. In other words, inclusion of a larger portion of MB in either fraction (POM or MF) is accompanied by inclusion of a larger portion of humus in that particular fraction and vice

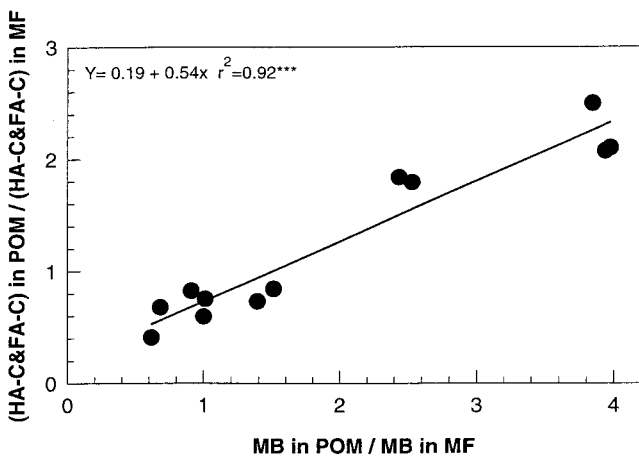


Fig. 7. Relationship of the amount of microbial biomass (MB) in particulate organic matter (POM) relative to mineral fraction (MF) to the amount of humic substances in POM relative to MF. Soils are air-dried and from greens sampled in 1997 and 1999. HA-C, humic acid carbon; FA-C, fulvic acid carbon. ***Both model and slope are significant at $P = 0.001$.

versa. Van Gestel et al. (1996) found a strong correlation ($r^2 = 0.77$) between clay content and MB concentration in aggregate-size fractions. Inclusion of organic C concentration in this relationship increased r^2 to 0.99. This may be explained by the fact that soil colloids with high cation exchange capacities, such as clay and humus, influence microbial survival by their greater capacity to adsorb to microbial cells (Amato and Ladd, 1992) in addition to the effect of slow nutrient release from recalcitrant components decomposed at low rates (Van Gestel et al., 1996). Thus, MB accumulation in POM would be favored by microbial growth on readily available substrates that become less accessible as POM fragments and the microbial cells themselves become coated with humus.

CONCLUSIONS

Soil MB increased as greens aged. This increase was due mainly to accumulation of POM. In younger greens (≤ 15 yr), a large portion of MB was located within the MF, whereas in older greens (> 15 yr), MB was preferentially associated with POM. Our study demonstrated that the spatial distribution of MB in the soil structure was determined in large part by the location of humus and vice versa. As microorganisms congregate near suitable food sources, metabolic by-products, i.e., humus, protect plant residues from further decomposition as both residues and microorganisms become coated with humus.

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