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Title: **Microbial biomass as a possible indicator of soil health**

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Abstract

Measuring soil health requires definition of contributing soil properties and system outputs. Soil quality indices have been employed in various studies, but they are complex and require multiple types of data. We analyzed papers with microbial biomass data in order to determine if this property could serve as an indicator of soil health. We found that microbial biomass carbon (MBC) was positively correlated with soil organic matter (OM). Moreover, MBC increased more steeply per unit of increasing OM for grassland soils, which have an abundant active fraction of OM, as compared to a lower slope for increasing MBC with OM of cropland soils, which are expected to contain mostly the passive fraction of OM. Thereby, MBC reports the activity of soil C for nutrient cycling. Microbial biomass carbon-to-nitrogen ratio (MB-C:N) was found to be negatively correlated with soil OM. This result was unexpected and likely relates to moisture, with fewer fungi and more bacteria under more moist conditions associated with higher OM. We had hoped to investigate the relationship between MB-C:N and the experimentally determined ratio of fungal-to-bacteria, but no correlations were found within our dataset. There is considerable variation among methods of estimating microbial biomass and microbial community composition. Literature review was broadly consistent with our metadata analysis, as follows. MBC offers considerable promise as an indicator of soil health and can be investigated in further practical studies in Manitoba. MBC gives insight into soil health by providing a signal that relates to the active fraction of organic matter, which is relevant to soil function and productivity. In contrast, measurement of OM alone may record only the passive fraction remaining in systems of reduced soil health. At the same time, further exploration of MB-C:N may yet offer insights for soil health as methods and baseline data become more established.

Introduction

Soil Quality was defined by Karlen et al. (1997) as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation." Some authors prefer the use of “fitness” instead of “capacity,” but this definition seems to have otherwise remained the generally accepted form for two decades.

A simpler and more often quoted definition is “the capacity of soil to function” (Karlen et al., 1997). This definition gives emphasis to a desired soil service. In agricultural scenarios, the crop or livestock production from the soil is the desired function, but others may be considered. Nine functions were given by Sombroek and Sims (FAO, 1995): production, environment for biota, atmospheric regulation, hydrologic function, storage, breakdown of waste and pollution, living space, heritage, and connective space.

There is some confusion about the use of the term “soil health,” especially in conjunction with the term “soil quality.” In agricultural terms, these terms are often used interchangeably. Soil health can be defined as “the soils fitness to support crop growth without becoming degraded” and without harming the environment (Acton and Gregorich, 1995). Using these terms interchangeably is not fully supported by the scientific community (Karlen et al., 1997). Some prefer the use of soil health to refer more to the biotic components of a soil, with soil quality encompassing abiotic soil properties (Anderson, 2003). This paper will follow the lead of Doran and Zeiss (2000) and consider the terms as synonyms.

Production is often used to gauge soil health in cropland. However, use of an indicator of soil health allows for prediction in advance of production being realized. Total organic carbon (TOC) is usually considered one of the most valuable indicators in agricultural crop production scenarios (Zornoza et al., 2015). For soil, TOC is interchangeable with organic matter (OM) by the equation: OM (%) = TOC (%) x 1.724 (Nelson and Sommers, 1996). Soil OM is correlated with a range of physical, chemical, and biological properties of soil, each of which could otherwise themselves be used as indicators. Respective examples of each category are soil pH, soil bulk density, and soil enzyme activities. Biological indicators can be expected to be the most useful for determining the health of the soil, because biological factors within the soil are influenced by the combined action of the physical and chemical properties of soil. Grasslands

rely on nutrient cycling more than do croplands, because they do not typically receive the same fertilization inputs. Going one step further, organic systems receive no inputs of inorganic fertilizer at all. It seems intuitive that the less inputs a system receives, the more likely it is that soil health will be best estimated by a biological indicator.

Metabolic quotient (qCO_2) is the ratio of CO_2 evolved to O_2 consumed. While TOC and pH are expected to be relevant for soil health in both cropland and grassland, MBC and soil qCO_2 relate to the microbial community. MBC and soil qCO_2 can thus be expected to be possible indicators of health for grasslands. North American tallgrass prairie ecosystems in their natural state tend to be N limited (Dell et al., 2005), and can therefore be expected to have fungi well represented (Bardgett et al., 1999). C:N ratio is 10 for fungi but only 5 for bacteria (Moore et al., 2000), and so we sought to determine if MB-C:N gave any insights into grassland soil health. In addition, we also investigated direct methods of measuring fungal-to-bacterial ratios, both for comparison to MB-C:N and for their potential as soil health indicators.

A common but complex method of objectively assessing the health of different soils is to form a soil quality index (SQI). Many different indicators are converted into dimensionless scores based on site-specific algorithmic relationships (Andrews et al., 2004). These scores can then be combined into a single index value, which can be considered to be an overall assessment of soil quality (Andrews et al., 2004). SQIs evaluate the soil quality across a range of soil characteristics. A commonly used SQI is the Soil Management Assessment Framework (SMAF), which was developed by Andrews et al. (2004). SMAF was used by numerous papers in the decade to follow (Erkossa et al., 2007; Shukla et al., 2006; Merril et al., 2013; Li et al., 2014). Calculation of SMAF can combine up to 84 indicators in an online format, but only thirteen example indicators were listed in the published form (Andrews et al., 2004): nematode maturity index, metabolic quotient (qCO_2), bulk density, soil test phosphorus, total organic carbon (TOC), microbial biomass carbon (MBC), potentially mineralizable nitrogen, pH, macroaggregate stability, available water capacity, electrical conductivity, and sodium adsorption ratio. Over the period the present review was undertaken, online calculation was not available (http://soilquality.org/tools/smaf_intro.html#).

Materials and Methods

Data Sources

Publications were collected using different combinations of the following keywords on Google Scholar and Ebscohost: soil, microbial, biomass, grassland, PLFA, community, and dynamics. Other papers were then found through the citations of the papers therein. Sources were included when they provided data for at least two from the following three ratios: (i) MBC as a percentage of TOC, (ii) MB-C:N, or (iii) estimation of the ratio of biomass for fungi-to-bacteria from methods other than microbial biomass. An attempt was made to focus on recent studies with a grassland focus, where possible. Most commonly, papers were excluded because they lacked data for one of organic carbon, organic matter, or microbial biomass nitrogen.

From 35 papers, the 338 samples investigated included 139 samples from grasslands, of which 59 were environments similar to those of Manitoba. Research in North America has been largely focused on croplands and natural areas, rather than grasslands and pasture. Much of the available research on grazing pasture that fulfills our criteria has been based in the United Kingdom, and is therefore under a maritime climate rather than a continental climate. In order to conduct an analysis of the data, samples were assigned to different ecosystems to enable comparison (Table 1). Seven ecosystem groups were used, which contain 87% of the samples. These ecosystems were: North American grassland, European grassland, temperate cropland, deciduous or mixed forest, coniferous forest, tropical grassland, and tropical forest. The remaining samples could not be combined into large enough groups to allow for useful analysis. Well aligned with our focus of interest, the two largest ecosystem groups were cropland, with 105 samples, and North American grassland, with 65 samples. Beyond these two groups, the variety of ecosystems provided a useful basis of comparison.

What follows is a brief summary of each paper included in the main dataset in order of their appearance in Table 1. We refer to the goal of each paper to illustrate alignment with the present review.

First, we consider European grassland. Using topsoil, Friedel and Scheller (2002) quantified the amino acid in hydrolysate of OM, litter OM, and extractant from the fumigation-extraction process. The soils in the study of Friedel and Scheller (2002) were from Germany and the Netherlands, and they included environments that were designed as barren, forested, cropland,

and grassland. Griffiths, Spilles, and Bonkowshi (2012) determined if nutrient cycles with the soil can be supplemented by agricultural fertilizer addition for a grazed grassland site in Ireland. Griffiths, Spilles, and Bonkowshi (2012) found increases following addition of P to the soil for MBC, microbial biomass N (MBN), and microbial biomass P. Frostegård and Bååth (1996) undertook one of the first studies to use analysis of phospholipid fatty acids (PLFAs) to determine bacterial biomass and fungal biomass in European soils. Frostegård and Bååth (1996) found the PLFA 18:2ω6 was well correlated with content of the fungal membrane sterol ergosterol, supporting the use of that PLFA as a measure of fungal biomass. Lovell, Jarvis, and Bardgett (1995) examined the effect of different N fertilization regimes on soil MBC and MBN for soils in SW England. Lovell, Jarvis, and Bardgett (1995) found that MBC appeared was not changed, but that numbers of culturable bacteria varied significantly among treatments. Subsequently, Lovell and Jarvis (1998) took soil samples from four different N fertilization regimes and measured the difference in microbial biomass between the samples and over time under controlled conditions. Lovell and Jarvis (1998) found higher MBC in soil from plots not fertilized and from plots with grass-clover treatments, compared to soils from plots with fertilized grass or clover alone. Bardgett et al. (1999) investigated microbial community composition, in term of fungi relative to bacteria, across a gradient of less-to-more intensively managed and fertile grasslands through the seasons. Bardgett et al. (1999) found that, in all seasons, highest values were found in plots not fertilized and not drained for MBC, MBN, total PLFAs and PLFA fungal-to-bacterial ratio. Bardgett and McAlister (1999) also found that ratios of fungi-to-bacteria were significantly higher in grasslands not fertilized, but that microbial respiration did not vary among management systems. Turner, Bristow, and Havgarth (2001) explored the effectiveness of ultraviolet absorbance of fumigated soil extracts as an alternative method of estimating soil microbial biomass. Using soil samples from across the UK and traditional fumigation extraction, Turner, Bristow, and Havgarth (2001) found that the UV method was well correlated with more traditional MBC estimation.

For North American grassland, Bailey, Smith, and Bolton (2002) determined fungal-to-bacterial ratios and MBC in five ecosystem types, and found higher MBC and higher fungal-to-bacterial ratios in restored prairie compared to cropland. Increased MBC in prairie is consistent with greater rooting densities and increased rhizodeposition, as compared to crop fields. Allison et al. (2005) studied the community structure of soil microbes following cessation of tillage

agriculture and the following succession into tallgrass prairie in a restoration chronosequence. Surprisingly, Allison et al. (2005) found that while fungal-to-bacteria ratios increased with OM in agricultural soils, the ratio decreased with increasing soil OM in prairie soils. Rice et al. (1994) studied the exposure of tallgrass prairie plots to increased atmospheric CO_2 treatments to determine its effect on the mount of C and N stored in soil OM and microbial biomass, as well as soil microbial activity. Banerjee et al. (2000) investigated how the seasons and how different pasture management systems can modify soil microbial and biochemical properties on a small pasture near Brandon, Manitoba. The data of Banerjee et al. (2000) suggested a link between stocking rate and grazing system on soil MBC and N mineralization, although the authors concluded that their study was too small to make any recommendations. Jordan et al. (1995) evaluated several microbial methods for their ability to indicate cropping history and soil quality on agricultural land. Methods were MBC, PLFAs, direct fungal and bacterial counts, and soil enzymes. Jordan et al. (1995) had sites for both farmland and prairie in Missouri, and they found soils were dominated by fungi. Shi, Yao, and Bowman (2006) analyzed the microbial properties of turfgrass soils across a chronosequence, along with comparisons to nearby native coniferous forest. Shi, Yao, and Bowman (2006) found that soil MBC and MBN both increased in older turfgrass systems, along with increases in microbial carbon-use efficiency and microbial nitrogen-use efficiency. The goal of Iyyemperumal, Israel, and Shi (2007) was to determine the effect of livestock camping near shade and water sources on the microbial and other properties of soil. Iyyemperumal, Israel, and Shi (2007) found that MBC and potential net N mineralization were greater in areas frequented by livestock and were significantly correlated with associated changes in total soil C and soil N. Zhang et al. (2005) wanted to determine what potential effect atmospheric warming would have on soil ecosystems. Results of Zhang et al. (2005) indicated that the enhancement of plant growth by warming, rather than the temperature increase itself, regulated the primary microbial response. Additionally, Zhang et al. (2005) found that warming increased the proportion of fungi relative to the rest of the microbial community. Carter (1991) investigated the effects of tillage on microbial biomass C and N, finding higher values in grassland and reduced tillage compared to plots with moldboard plowing. More important for our focus, Carter (1991) found that compared to tilled soil, reduced tillage increased the MBC level per unit soil OM. The goal of Corre, Schnabel, and Stout (2002) was to quantify rates of N-cycling processes within grassland under negligible management, and to investigate the role of

slope and climate on the spatial and seasonal variations of these processes. Corre, Schnabel, and Stout (2002) found that nitrate immobilization was highest when ammonium was insufficient to meet microbial demand, and that nitrate was rapidly produced and consumed, resulting in a high turnover despite low pool size at any one time.

Chen et al. (2015) studied the impact that thinning of a Chinese fir tree plantation had on microbial communities. Chen et al. (2015) found that MBC and MBN were highest after intense thinning, but that microbial functional diversity was higher under low intensity thinning. Xu et al. (2013) conducted a study to estimate the concentrations, stoichiometry, and storage of soil microbial C, N, and P, both globally and across different biomes. The global scale and broad biome categorization of Xu et al. (2013) mostly precluded incorporation of their study into our review. Sparling et al. (1994) compared microbial biomass C, N, and P, along with other soil properties, across native forest, land cleared for pasture, and land cleared for plantations. Sparling et al. (1994) found no decline in soil and microbial C, N, and P concentrations, or in macro-aggregate stability, in the cleared ecosystems compared to native forest. Allen and Schlesinger (2004) subjected forest floor and soil cores samples from North Carolina loblolly pine forests to additions of C, N, or P in solution form. The effects of these solutions were measured in the MBC and soil respiration, in order to determine the extent of nutrient limitation in the samples. Wang et al. (2004) investigated the distribution of MBC and MBN in five different vegetation systems in Eastern China. Of their systems, we used data from Chinese fir, citrus orchard, and rice plots.

Soil health of cropland has been intensively studied. Johnson et al. (2004) determined that electrical conductivity could be used as a cost effective reference for relating data collected at different scales to soil health. Johnson et al. (2004) took several different soil properties, including MBC, and grouped them together into different categories of soil electrical conductivity. Klose and Tabatabai (2000) studied the urease activity of microbial biomass in soils. Moore, Klose, and Tabatabai (2000) studied the effect of different crop rotation and N fertilization regimes on soil MB C and MBN. Moore, Klose, and Tabatabai (2000) found that vegetation had a significant effect on microbial biomass, with the multi-cropping systems having the highest microbial biomass contents, and the continuous corn or soybean rotations having the lowest. Fertilization was determined to have no effect on microbial biomass (Moore, Klose, and Tabatabai, 2000). Barbhuiya et al. (2004) studied the dynamics of soil microbial biomass and its

relation to soil OM and nutrient fluxes in sites of varying disturbance. Barbhuiya et al. (2004) found that a frequently cultivated site had the lowest labile fraction of OM and large variation in overall microbial biomass between different seasons. Balota et al. (2003) investigated the effect of different crop rotations and tillage regimes on soil MBC. Balota et al. (2003) found that microbial biomass was higher under no tillage systems and that soil OM under no tillage systems provided higher levels of more labile C than conventional tillage systems. Mbuthia et al. (2015) used SMAF to assess the health of soils using conservation agricultural practices. Mbuthia et al. (2015) found that TOC, soil quality, and yields were all higher under conservation practices.

Sugihara et al. (2015) investigated the effect of vegetation upon seasonal fluctuations of soil MBC, along with other soil properties, within tropical forest and savanna ecosystems in Central Africa. Savanah was found to be an N-limited ecosystem, and that overall soil pH rather than residues controlled the soil microbial communities. Agbenin and Adeniyi (2005) analyzed five managed pasture field crops for their effect on soil OM, MBC, and MBN, for the purposes of rehabilitating degraded or unproductive native grassland. Arunachalam et al. (1996) investigated the impact of disturbance in a tropical forest in India on soil community structure and nutrient status. Minimal changes were found in natural vegetation gaps, but Arunachalam et al. (1996) found that their logged site and a disturbed soil pile had significantly reductions in all functional parameters. Arunachalam and Arunachalam (2000) then studied the effect of treefall gaps size and soil properties on MBC in an undisturbed tropical forest in India. Arunachalam and Arunachalam (2000) found that the resulting microclimate variations had a significant effect on MBC and related nutrient cycling.

Dataset Methodological Summary

There are four different ways of measuring soil MB C and MBN in the reviewed papers. These methods were: the fumigation extraction (FE) method (Vance *et al.*, 1987), the fumigation incubation (FI) method (Jenkinson, 1966, 1988), the substrate induced respiration (SIR) method (Anderson & Domsch, 1978), and the microwave soil extraction method (Islam & Weil, 1998). For MBC, fumigation extraction was by far the most popular method of measurement, used for 65% of the samples, followed by 11% using FI, 9% using SIR, and 1% using microwave soil extraction. A further 6% either had their method unlisted or were composites, and 8% of the total samples did not include MBC. For MBN, FE was again the most popular method and was used

for 62% of the samples, followed by 13% FE, and 1% with microwave soil extraction. A further 5% either had their method unlisted or were composites, and 18% of the total samples did not included MBN.

Four different methods of calculating or reporting relative concentrations of fungi and bacteria were found, although details varied greatly within each method. These methods were counts of cultured colonies, PLFAs, respiration analysis with selective inhibitors, and biomass determined from a colony count. Papers which reported concentrations in number of colonies per weight of soil varied in substrate used. PLFA analyses varied in which fatty acids were chosen to represent fungal or bacterial concentration, and whether the total PLFAs was larger than the sum of bacterial and fungal markers, or equal to that sum. Respiration analysis with selective inhibitors is functionally the SIR method, but with application of bactericides or fungicides to determine which group is contributing to respiration. Use of SIR in this regard varied with different substrates and with different antibiotics.

Results and Discussion

Ecosystems were compared using one-way analysis of variance for soil pH, OM, MBC, the ratio MBC:TOC, and MB-C:N ratio (Table 2). As expected, the pH in the soil of coniferous forest was lower than that of either cropland or grasslands. Coniferous forest has low pH, because (i) conifers occur naturally on sandy substrates that are low in calcium and poorly buffered, and (ii) conifer needle litter contains resins that lead to acidification of soil. The average pH values we collected for cropland and N. American grassland are both moderate and close to pH = 6. The soil pH values of the European grassland were lower, which could be due to proximity to industry. The majority of European grassland samples were taken from British soils, which are sufficiently acidic to require periodic liming in many cases.

Soil OM is lower in cropland compared to European grassland, as expected. The loss of soil OM under cultivation is well documented and is attributed to soil aeration and reduced residue inputs (Burke et al., 1989; Brady and Weil, 2002). OM was highest in coniferous forest soil, because litter inputs persist under conditions that are cool and moist, limiting decomposition of soil OM in coniferous forest (Swift et al., 1979; Anderson, 1991).

MBC was highest in European grassland, and also high in N. American grassland, but decidedly lower in cropland. Loss of MBC in cropland relates to the support of the microbial population by root exudates, which are higher with the greater root biomass of grasslands (Agbenin and Adeniyi, 2005). Aridity in tropical grassland limits productivity and, thereby, the residue inputs fueling microbial biomass in such systems (Swift et al., 1979; Grace et al., 2006). Temperate forests were intermediate between temperate and tropical grasslands, a result which follows with lower root exudates in forested ecosystems versus grasslands. MBC evidently provides a sensitive means to discern among ecosystem types, as seen in the pronounced significant differences.

MBC as percentage of TOC is significantly lower for cropland compared to grasslands. This reduction means that not only was MBC in cropland lower because of less soil OM in croplands, but also that proportionately less soil OM in cropland is able to support microbial biomass. When soil comes under cultivation, removal of plant residues causes decline in the active portion of organic matter (Brady and Weil, 2002). The soil OM that remains in cultivated cropland soil is predominantly passive, and cycles with the rest of the soil ecosystem on a

timescale of centuries to millennia. The sensitivity of MBC to this loss of the active fraction of soil OM demonstrates that MBC is a potentially more valuable method of measuring soil health opposed to measuring soil OM alone. The biological component of soil is responsible for nutrient cycling and is therefore most relevant for the evaluation of the health of any soil ecosystem (Ingham et al., 1985).

Given their greater relevance to Manitoba systems, N. American grassland and cropland were then considered alone to evaluate correlations as follows. No significant correlations were found when comparing pH to three properties: OM, MBC, or MB-C:N ratios. Microbial biomass increased significantly in response to increasing organic matter in both North American grassland (Table 3) and cropland (Table 4). MBC increases from about 300 $\mu g\ g^{-1}$ at 25 $g\ kg^{-1}$ organic matter to approximately 1700 $\mu g\ g^{-1}$ at 120 $g\ kg^{-1}$ OM in North American grassland (Fig. 1). In contrast, a more modest increase in MBC was seen for increments in OM in cropland (Fig. 2). To illustrate the comparison, the slope of increase of MBC in response to OM elevation was almost three-fold higher in North American grassland at 14.5 $(\mu g\ g^{-1})/(g\ kg^{-1})$ compared to 5.6 $(\mu g\ g^{-1})/(g\ kg^{-1})$ for cropland. The mechanism for steeper increase in microbial biomass per unit of organic matter in grassland as compared to cropland must relate to a difference in the quality of the organic matter between the two systems. Conversion of grassland to cropland is reported to differentially eliminate the active fraction of organic matter formerly therein, so that the remaining OM in cropland is more passive and cannot support microbe development in the profuse way inherent for natural grassland systems.

The ratio MB-C:N seems to be a poor basis of comparison between ecosystems (Table 2). MB-C:N values indicated a broad spread of different blends of fungi and bacteria, and the large standard deviation values imply a large variation in MB-C:N, even within ecosystem types. MB-C:N decreased significantly in response to increasing organic matter in N. American grassland (Table 5). Microbial biomass C:N decreases from about 10.5 at 30 $g\ kg^{-1}$ OM to approximately 3.0 at 105 $g\ kg^{-1}$ organic matter (Fig. 3). Grassland soil ecosystems are expected to have abundant fungi, and grassland soils have high OM from root biomass. Therefore, reduction of C:N ratio with increasing OM goes against expectations. Possibly, this negative relationship may be driven by moisture. Water tends to increase productivity of grassland ecosystems, which would drive increasing OM, but at the same time limit the aerobic respiration of fungi, driving the microbial community more towards anaerobic bacteria.

Conclusion

Across a range of studies from different research groups in different regions, the remarkable outcome from our meta-analysis of data from the literature is a consistent positive relationship between MBC and soil OM. In particular, we see the relationship is different for contrasting ecosystem types: MBC increases faster per unit of OM in grassland, but more slowly in cropland. This outcome testifies that MBC is of value as an indicator of soil health, because we attribute degradation of soil health from the loss of active fraction of OM in cropland soils. Data are highly variable for MBN, and so findings are more uncertain at this stage for the utility of MBN and of MB-C:N ratio. Further data for MBN with improved methods will hopefully clarify these points. Unfortunately, few studies hitherto have investigated MBC in relation to grazing management. Experimental evaluation of the possible application of MBC as an indicator of soil health in grazing systems is clearly warranted, given the strength of signal from MBC in comparison of grassland, crop, and other systems.

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Table 1. Source and location of ecosystem data.

Ecosystem	Location	Number of Samples	Authors	Year
European Grassland	Germany	1	Friedel, J. K., & Scheller, E.	2002
European Grassland	Ireland	6	Griffiths, B. S., Spilles, A., & Bonkowski, M.	2012
European Grassland	Sweden	3	Frostegård, Å., & Bååth, E.	1996
European Grassland	UK	5	Lovell, R. D., Jarvis, S. C., & Bardgett, R. D.	1995
European Grassland	UK	4	Lovell, R. D., & Jarvis, S. C.	1998
European Grassland	UK	4	Bardgett, R. D., Lovell, R. D., Hobbs, P. J., & Jarvis, S. C.	1999
European Grassland	UK	4	Bardgett, R. D., & McAlister, E.	1999
European Grassland	UK	29	Turner, B. L., Bristow, A. W., & Haygarth, P. M.	2001
North American Grassland	Illinois, USA	2	Bailey, V. L., Smith, J. L., & Bolton, H.	2002
North American Grassland	Illinois, USA	9	Allison, V. J., Miller, R. M., Jastrow, J. D., Matamala, R., & Zak, D. R.	2005
North American Grassland	Kansas, USA	12	Rice, C. W., Garcia, F. O., Hampton, C. O., & Owensby, C. E.	1994
North American Grassland	Manitoba, Canada	8	Banerjee, M. R., Burton, D. L., WP (Paul) McCaughey, & Grant, C. A	2000
North American Grassland	Missouri, USA	3	Jordan, D., Kremer, R. J., Bergfield, W. A., Kim, K. Y., & Cacnio, V. N.	1995
North American Grassland	North Carolina, USA	8	Shi, W., Yao, H., & Bowman, D.	2006
North American Grassland	North Carolina, USA	5	Iyyemperumal, K., Israel, D. W., & Shi, W	2007
North American Grassland	Oklahoma, USA	12	Zhang, W., Parker, K. M., Luo, Y., Wan, S., Wallace, L. L., & Hu, S.	2005
North American Grassland	PEI, Canada	3	Carter, M. R	1991
North American	Pennsylvania, USA	3	Corre, M. D., Schnabel, R. R., & Stout, W. L.	2002

Grassland				
Temperate Coniferous Forest	Alabama, USA	2	Bailey, V. L., Smith, J. L., & Bolton, H.	2002
Temperate Coniferous Forest	China	3	Chen, X. L., Wang, D., Chen, X., Wang, J., Diao, J. J., Zhang, J. Y., & Guan, Q. W.	2015
Temperate Coniferous Forest	Germany	2	Friedel, J. K., & Scheller, E.	2002
Temperate Coniferous Forest	Global Composite	1	Xu, Xiaofeng, Peter E. Thornton, and Wilfred M. Post	2013
Temperate Coniferous Forest	New Zealand	4	Sparling, G. P., Hart, P. B. S., August, J. A., & Leslie, D. M.	1994
Temperate Coniferous Forest	North Carolina, USA	3	Allen, A. S., and W. H. Schlesinger	2004
Temperate Coniferous Forest	North Carolina, USA	2	Shi, W., Yao, H., & Bowman, D.	2006
Temperate Coniferous Forest	Southeastern China	3	Wang F.E, Chen Y.X, Tian G.M, Kumar S., He Y.F, Fu Q.L, Lin Q	2004
Temperate Coniferous Forest	Sweden	3	Frostegård, Å., & Bååth, E.	1996
Temperate Coniferous Forest	Washington, USA	2	Bailey, V. L., Smith, J. L., & Bolton, H.	2002
Temperate Cropland	Colorado, USA	4	Johnson, C. K., Wienhold, B. J., Doran, J. W., Drijber, R. A., & Wright, S. F.	2004
Temperate Cropland	Germany	3	Friedel, J. K., & Scheller, E.	2002
Temperate Cropland	Great Plains, USA	10	Klose, S., & Tabatabai, M. A.	1999
Temperate Cropland	Illinois, USA	2	Allison, V. J., Miller, R. M., Jastrow, J. D., Matamala, R., & Zak, D. R.	2005
Temperate Cropland	Iowa, USA	35	Moore, J. M., Klose, S., & Tabatabai, M. A.	2000
Temperate Cropland	Missouri, USA	5	Jordan, D., Kremer, R. J., Bergfield, W. A., Kim, K. Y., & Cacnio, V. N.	1995
Temperate Cropland	Netherlands	1	Friedel, J. K., & Scheller, E.	2002
Temperate Cropland	North-east India	2	Barbhuiya, A. R., Arunachalam, A., Pandey, H. N., Arunachalam, K., Khan, M. L., & Nath, P. C.	2004
Temperate Cropland	PEI, Canada	10	Carter, M. R	1991

Temperate Cropland	Southeastern China	3	Wang F.E, Chen Y.X, Tian G.M, Kumar S., He Y.F, Fu Q.L, Lin Q	2004
Temperate Cropland	Southern Brazil	18	Balota, E. L., Colozzi-Filho, A., Andrade, D. S., & Dick, R. P.	2003
Temperate Cropland	Sweden	1	Frostegård, Å., & Bååth, E.	1996
Temperate Cropland	Tenessee, USA	9	Mbuthia, L. W., Acosta-Martínez, V., Debryun, J., Schaeffer, S., Tyler, D., Odoi, E., ... & Eash, N.	2015
Temperate Cropland	Washington, USA	2	Bailey, V. L., Smith, J. L., & Bolton, H.	2002
Temperate Deciduous Forest	Global Composite	1	Xu, Xiaofeng, Peter E. Thornton, and Wilfred M. Post	2013
Temperate Deciduous Forest	Southeastern China	3	Wang F.E, Chen Y.X, Tian G.M, Kumar S., He Y.F, Fu Q.L, Lin Q	2004
Temperate Deciduous Forest	Sweden	3	Frostegård, Å., & Bååth, E.	1996
Temperate Mixed Forest	Global Composite	2	Xu, Xiaofeng, Peter E. Thornton, and Wilfred M. Post	2013
Temperate Mixed Forest	New Zealand	4	Sparling, G. P., Hart, P. B. S., August, J. A., & Leslie, D. M.	1994
Tropical Grassland	Eastern Cameroon	1	Sugihara, S., Shibata, M., Ze, A. D. M., Araki, S., & Funakawa, S.	2015
Tropical Grassland	Northern Nigeria	5	Agbenin, John O., and Tomilayo Adeniyi	2005
Tropical Rainforest	Eastern Cameroon	1	Sugihara, S., Shibata, M., Ze, A. D. M., Araki, S., & Funakawa, S.	2015
Tropical Rainforest	Global Composite	1	Xu, Xiaofeng, Peter E. Thornton, and Wilfred M. Post	2013
Tropical Rainforest	North-east India	3	Arunachalam, A., Maithani, K., Pandey, H. N., & Tripathi, R. S	1996
Tropical Rainforest	North-east India	14	Arunachalam, A., & Arunachalam, K.	2000
Tropical Rainforest	North-east India	4	Barbhuiya, A. R., Arunachalam, A., Pandey, H. N., Arunachalam, K., Khan, M. L., & Nath, P. C.	2004

Table 2. Soil and microbial properties summarized as mean ± s.d. for ecosystem categories.

Ecosystem	**Soil pH**	**Soil OM ($g\ kg^{-1}$)**	**MBC ($\mu g\ g^{-1}$)**	**MBC:TOC (%)**	**MB-C:N**
N. American grassland	6.2 ± 0.6 c	61 ± 27 ab	810 ± 445 bc	2.5 ± 0.8 bc	11.8 ± 14.6 a
European grassland	5.4 ± 0.6 b	119 ± 53 bc	1435 ± 520 d	3.1 ± 1.2 c	7.4 ± 1.8 a
Cropland	5.9 ± 0.9 c	38 ± 16 a	295 ± 180 a	1.3 ± 0.6 a	8.2 ± 5.6 a
Deciduous/mixed forest	5.2 ± 1.0 ab	64 ± 51 abc	418 ± 328 ab	1.3 ± 0.3 a	7.0 ± 3.0 a
Coniferous forest	4.5 ± 0.6 a	134 ± 235 c	545 ± 460 ab	1.7 ± 0.6 ab	7.0 ± 2.3 a
Tropical grassland	5.6 ± 0.2 bc	27 ± 8 a	390 ± 70 ab	2.6 ± 0.9 bc	6.5 ± 2.2 a
Tropical rainforest	5.1 ± 0.4 ab	65 ± 32 abc	1020 ± 570 c	3.1 ± 1.5 c	6.8 ± 3.4 a

Table 3. Linear regression analysis of variance for the plot of microbial biomass carbon as dependent variable against soil organic matter for North American grassland, as plotted in Fig. 1.

Source	DF	SS	MS	F	P
Regression	1	5633694	5633694	76.65	<0.001
Residual	36	2645941	73498		
Total	37	8279636			

Table 4. Linear regression analysis of variance for the plot of microbial biomass carbon as dependent variable against soil organic matter for cropland, as plotted in Fig. 2.

Source	DF	SS	MS	F	P
Regression	1	747421	747421	107.70	<0.001
Residual	91	631500	6940		
Total	92	1378921			

Table 5. Linear regression analysis of variance for the plot of microbial biomass carbon-to-nitrogen ratio as dependent variable against soil organic matter for North American grassland, as plotted in Fig. 3.

Source	DF	SS	MS	F	P
Regression	1	111.271	111.271	12.69	0.002
Residual	24	210.505	8.771		
Total	25	321.776			

Fig. 1. Microbial Biomass Carbon (MBC) plotted against N. American grassland soil organic matter (OM). Linear regression y=6.1+14.5x was significant (P<0.001, Table 3) for n=38 and with r^2=0.68.

Fig. 2. Microbial Biomass Carbon (MBC) plotted against Cropland soil organic matter (OM). Linear regression y=52.3+5.6x was significant (P<0.001, Table 4) for n=93 and with r^2=0.54.

Fig. 3. Microbial Biomass Carbon-to-Nitrogen ratio (CN) plotted against N. American grassland soil organic matter (OM). Linear regression y=10.7-0.074x was significant (P=0.002, Table 5) for n=26 and with r^2=0.35.

Fig. 1. Microbial biomass carbon (MBC) plotted against N. American grassland soil organic matter (OM). Linear regression y=6.1+14.5x was significant (P<0.001, Table 3) for n=38 and with r^2=0.68.

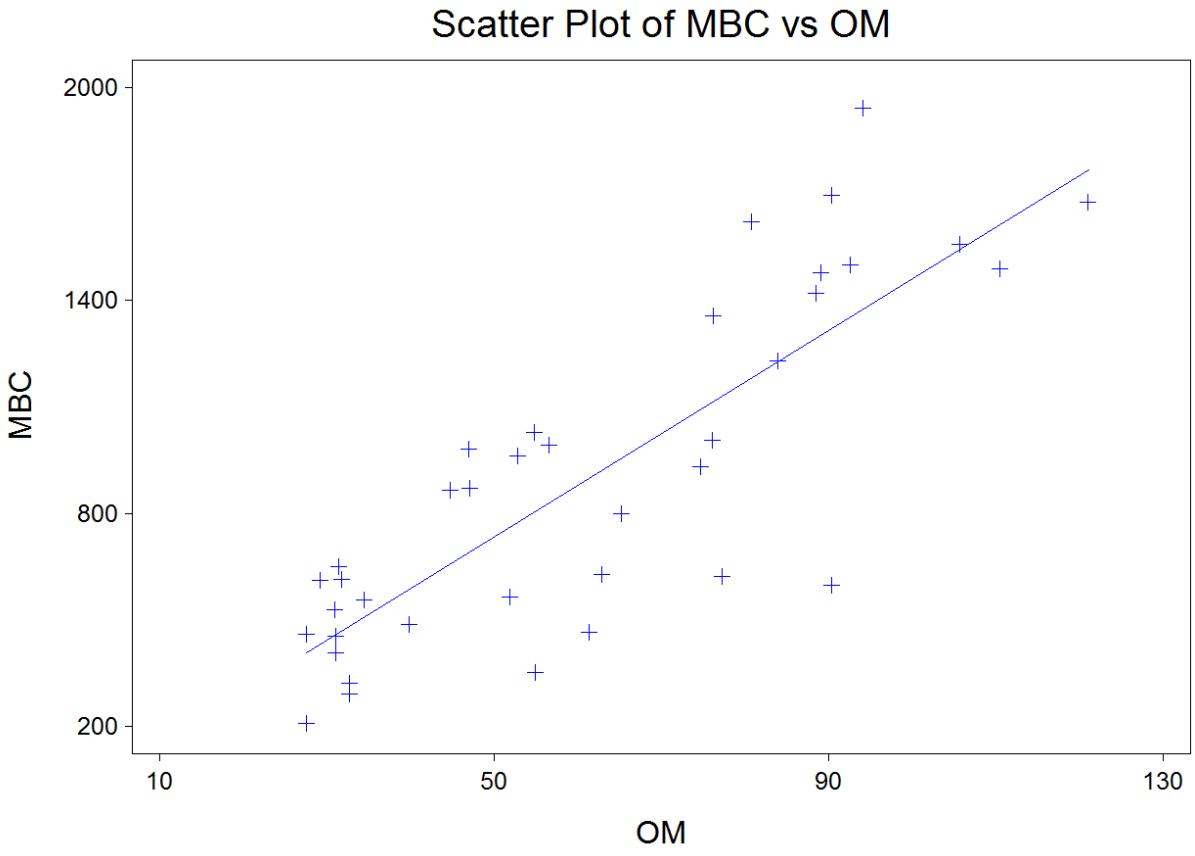
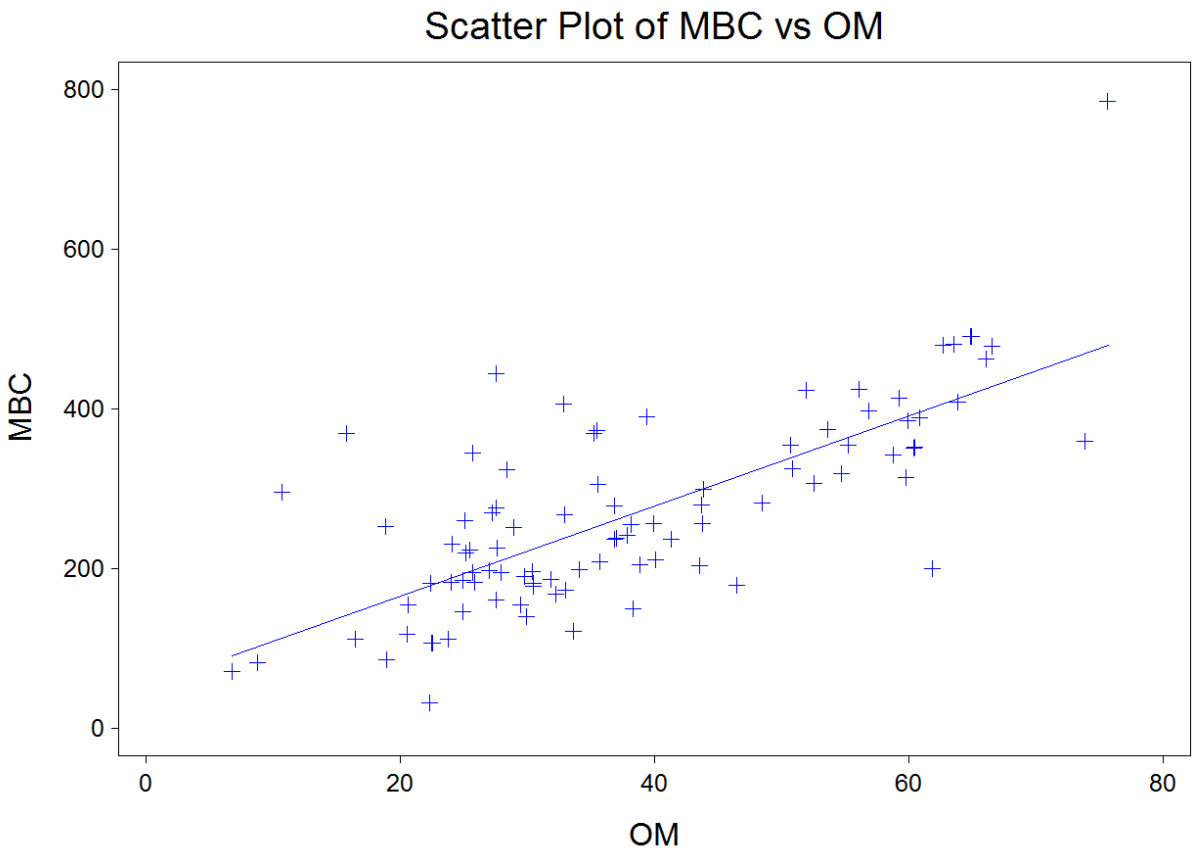


Fig. 2. Microbial biomass carbon (MBC) plotted against cropland soil organic matter (OM). Linear regression y=52.3+5.6x was significant (P<0.001, Table 4) for n=93 and with r^2=0.54.



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