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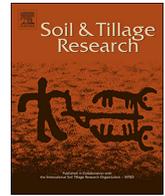
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Changes in soil microbial biomass with manure application in cropping systems: A meta-analysis

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ABSTRACT

Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) are important indices of soil bio-fertility. While intensively managed cropping systems can reduce microbial biomass, application of manure is a potential way to rebuilt microbial biomass and improve soil functions. However, the responses of SMBC and SMBN to manure application relative to mineral fertilizers (NPK) in Chinese cropping systems remains unclear. We conducted a meta-analysis based on 103 peer-reviewed publications with 1448 paired observations to identify the degree to which climate types, soil properties and agricultural managements regulate the responses of microbial biomass to manure amendment relative to NPK. The results indicated that manure application increased SMBC, SMBN, SMBC/soil organic carbon (SOC) and SMBN/soil total nitrogen (TN) by 40%, 55%, 16% and 21%, respectively, across all the observations compared to NPK. SMBC/SMBN under manure amendment (6.58 in average) was lower than that in NPK (7.86 in average). Manure-related factors, e.g. manure types, duration of application, manure-C and N input rates, were the strongest regulators of the response of microbial biomass. Soil properties and climates also contributed to considerable degrees of variation in microbial biomass response based on variance partitioning analysis (VPA). Results of the random forest (RF) models showed that manure type, application rate (manure-C and N input) as well as soil initial properties (SOC, TN and clay contents) were likely the predominant factors controlling the response of microbial biomass to manure application. Our study indicates that manure application can be an effective way to restore the loss of microbial biomass due to intensive application of NPK, yet variations in response are determined by specific manure type, application rate, as well as local conditions of climate and inherent soil properties.

1. Introduction

In order to meet the challenge of feeding 22% of the global population with only 7% of its arable land area, mineral fertilizers have been intensively applied in agricultural system for decades in China, which is now the largest consumer of mineral fertilizers in the world (Liu and Diamond, 2005; Zhang et al., 2015). Although the grain yield has been highly elevated by application of mineral fertilizers, there are growing evidences that unlimited application of mineral fertilizers has incurred substantial environmental risks, including severe disruption of soil physical properties (Idkowiak, 2004), increase in greenhouse gas emissions (Zhang et al., 2013) and nutrient run-off (Miao et al., 2011), and disturbance in soil microbial community (Postma-Blaaauw et al.,

2010; Qiu et al., 2016). In consequence, in 2015, the Ministry of Agriculture and Rural Affairs of the People's Republic of China has announced a 'Zero Increase Action Plan' for the utilization of mineral fertilizer by 2020 across the nation (Liu et al., 2015). Implement of alternative management to replace mineral fertilizer is hence necessary to accomplish yield demand meanwhile mitigate environmental costs.

Soil microbes are crucial component in a large number of important ecosystem processes, including decomposition (Gessner et al., 2010), nutrient acquisition (Perelo and Munch, 2005; Perelo et al., 2006a,b), carbon (C) and nitrogen (N) cycling (Manzoni and Porporato, 2009) as well as soil formation (Rillig and Mummey, 2006) and C sequestration (Six et al., 2006). Soil microbial biomass, often measured as microbial biomass carbon (SMBC) and nitrogen (SMBN), is a key indicator of soil

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biological traits (Acosta-Martinez et al., 2008; Xu et al., 2008). Declines in microbial biomass in arable soils relative to unmanaged ecosystems are often observed because of the decreases in plant C input and hence C availability in agricultural systems (Fierer et al., 2009). Excess application of mineral fertilizer can further introduce negative effect on soil microbial biomass due to soil acidification, changes in community composition, and various chemical interactions (Treseder, 2008; Maly et al., 2009). A 12–48% decrease in SMBC and SMBN by mineral fertilizer application related to no fertilizer or the initial values of the experiments has been reported (Bittman et al., 2005; Maly et al., 2009; Qiu et al., 2016). In addition, recent meta-analyses have suggested that the decrease in SMBC and SMBN resulted from increased mineral N inputs ranged from 5.8% to 20% in unmanaged ecosystems (Treseder, 2008; Liu and Greaver, 2010; Lu et al., 2011). In contrast to conventional mineral fertilizer, organic amendments such as manure can increase C availability for soil microbes by delivering high rate of exogenous C into soil, which can be beneficial for enhancing microbial biomass compared with mineral fertilizer application only (Jangid et al., 2008; Neufeld et al., 2017). Pan et al. (2009) demonstrated that manure application increased SMBC and SMBN by 13% and 49% compared with application of mineral fertilizer. Therefore, manure amendment could be an alternative solution for the problems of excessive application of mineral fertilizer meanwhile improving soil bio-fertility and maintaining grain yield (Li et al., 2015; Pan et al., 2009).

Although c.a. 4.6 billion tons of manure is produced from livestock sector each year, only a small proportion were applied to arable fields due to high labor costs of collecting, transporting and applying manure to cropland, disconnection and lack of appropriate storage and handling facilities (Niu and Ju, 2017; Ma et al., 2010; Ju et al., 2005). Given the key role of soil microbes in regulating multiple ecosystem processes and the potential of manure to rebuilt microbial biomass and associated ecosystem functions, it is of great importance to better understand the magnitude of microbial biomass responses to manure. Previous study has suggested that after 10 years organic amendment soil microbial biomass could be recovered to a near pre-cultivation level (Wu et al., 2004), but responses of microbial biomass can be specific depending on other management practice, manure type and abiotic factors such as soil properties and climate (Liang et al., 2011; Zhen et al., 2014; Deng et al., 2006; Gunapala and Scow, 1998; Jangid et al., 2008). There are also considerable uncertainties in the magnitude of microbial biomass responses to manure relative to mineral fertilizer under various environmental and management conditions. Up to now, there is however no systematic synthesis of the independent single researches to compare the effect of manure application on SMBC and SMBN with mineral fertilizers that encompassing a range of agronomic managements, soil types and climate conditions in China (Liang et al., 2011; Zhen et al., 2014; Kallenbach and Grandy, 2011; Sun et al., 2014). It is therefore pivotal to understand the relationships between the effect of manure application on microbial biomass and various management and environmental factors in order to improve microbial biomass and restore functions in intensive agricultural systems that are routinely fertilized with mineral fertilizers at a national scale.

Thus, in the present study, we performed a comprehensive meta-analysis to integrate previously published results on SMBC and SMBN responses to manure amendment relative to agricultural systems that receive only mineral fertilizers. Comprehensive information of management and environmental conditions that have potential influence on microbial biomass were extracted to characterize how SMBC and SMBN changes after manure application across major crop systems in China. These variables were further categorized into three explanatory factors, i.e. soil factors, farming practices and climate, and their contributions to the variations in SMBC and SMBN responses to manure were partitioned by variance partitioning analysis (VPA). Furthermore, a random forest (RF) model was used to gain a mechanistic understanding of the drivers of the variations in SMBC and SMBN responses to manure application. Altogether the present study aimed to provide a predictive

and mechanistic understanding of the relative improvement in microbial biomass by manure application across major intensively managed cultivation systems in China.

2. Materials and methods

2.1. Data collection

To fully cover the research on microbial biomass in Chinese soils, our meta-analysis was based on peer-reviewed articles published between 1990 and September 2017 using the online database Web of Science (<http://apps.webofknowledge.com/>) and the China Knowledge Resource Integrated Database (<http://www.cnki.net/>) for studies that published in Chinese. Manure from different sources has processing methods from fresh manure to compost and different nutrient contents. In this study, we considered the following sources: swine (SW), sheep (SP), poultry (PL), cattle (CT), horse (HS) and farmyard manure (FYM). SOC and N content in SW is medium but it is higher in SP (<http://www.cnoa.com/>). PL has a low C/N ratio and high N and cellulose concentrations (Mubarak et al., 2010) and the PL typically has high levels of NH_4^+ and NO_3^- (Wang et al., 2004; Bernal et al., 2009). Organic matter is more stable in CT (Velthof et al., 2000). And CT has a medium C/N ratio, high dry matter, organic N contents and low NH_4^+ (Lupwayi et al., 2005). HS has high cellulose content (<http://www.cnoa.com/>). Generally, FYM is a mixture of human and animal waste and household garbage and so its nutrient content varies from year to year and the nutritional composition and physicochemical properties of farmyard manure are poor (Liu et al., 2010). Keywords that used in the literature retrieval were *manure* with its sources, SMBC, SMBN, SOC and TN. Crop types covered wheat, maize, rice, soybean and oilseed rape with different crop rotation patterns (wheat-maize, wheat-rice, wheat-soybean, wheat-rape, rice-rape, rice-rice, maize, wheat, soybean, rice-rice-wheat, etc.). The following criteria were used to select publications: 1) all published results should be based on field experiments with a minimum of three replications for each treatment, and 2) at least two types of treatments must be involved: (a) balanced application of mineral N, phosphorus (P) and potassium (K) fertilizers (NPK) and (b) manure amendment alone (M) or combined with mineral fertilizers (NPKM). We found a total of 103 publications that met the above criteria. We focused on the dependent variables of SMBC, SMBN, SMBC/SOC and SMBN/TN (focused variables). Because samples should be independent in a meta-analysis, only the final observed values of the focused variables at one site were used if repeated measurements happened (Gurevitch and Hedges, 1999; Tian et al., 2015). Within these publications we obtained 410 pairs of observations reported on SMBC, 332 pairs on SMBN, 394 pairs on SMBC/SOC and 315 pairs on SMBN/TN to use for the meta-analysis.

Ten variables that are well known for affecting microbial biomass were identified from each original study and compiled into the database for analyses (Table 1; Appendix S1.). For instance, longitude and latitude of experimental location can reflect climate types, which are primary factors influencing microbial growth. Soil properties such as soil acidity (Fierer and Jackson, 2006), soil texture (Muller and Hoper, 2004) and initial TN and SOC contents (Kallenbach and Grandy, 2011) can have strong influence on microbial community. Other variables such as types of applied manure, duration of the practice (Maul and Drinkwater, 2010) and land use types (Jangid et al., 2008) that would influence microbial growth and their activities were also included. These ten variables were further classified into different levels (Table 1) to assess the relative changes of the focused variables at each level. Experimental durations and rates of manure-C and N inputs were divided different levels to make the variable distributed as evenly as possible. The classification of SOC and TN was based on the standard operating procedure of the second national soil census (NSCO, 1979). In those studies that SOM content was reported instead of SOC, SOM was converted to SOC using the van Bemmelen factor of 0.58 (Bemmelen,

Table 1

A list of variables and the levels of each variable tested for significance as predictors in SMBC, SMBN, SMBC/SOC and SMBN/TN response in the meta-analysis.

Variable	group	explanation
Climate	STM	Subtropical monsoon climate
	NTM	Temperate monsoon climate
	NTC	Temperate continental climate
Type of manure	SW	Swine manure, composted and uncomposted
	SP	Sheep manure, composted and uncomposted
	PL	Poultry manure, composted and uncomposted
	FYM	Farm yard manure composted and uncomposted
	CT	Cattle manure, compost and uncomposted
Experimental duration	HS	Horse manure, compost and uncomposted
	> 30	The number of years manure input
	25–30	
	15–25	
5–15		
Land use type	< 5	
	P	Paddy soil
	U-P	Upland-paddy soil
	U	Upland soil
N rate	> 200	Rate of manure-N input (kg N ha ⁻¹ yr ⁻¹)
	100–200	
C rate	< 100	Rate of manure-C input (kg C ha ⁻¹ yr ⁻¹)
	> 4000	
	2000–4000	
TN	< 2000	
	> 2.0 (rich)	Soil nitrogen content in surface soil (0–20 cm) g kg ⁻¹
	1.5–2.0 (medium)	
	0.75–1.5 (less)	
< 0.75 (poor)		
SOC	> 20 (rich)	Soil carbon content in surface soil (0–20 cm) g kg ⁻¹
	12–20 (medium)	
	6–12 (less)	
	< 6 (poor)	
Soil texture	Sandy	Sand content > 50%; Clay content < 30%
	Loam	Sand content 20–50%; Clay content < 30%
	Clay	Sand content < 20%; Clay content > 30%
Soil pH	> 8 (alkaline soils)	Soil pH in surface soil (0–20 cm)
	6–8 (neutral soils)	
	< 6 (acid soils)	

1890). The division of soil texture was based on the Chinese soil texture classification (Xiong and Chen, 1986).

2.2. Meta- analysis

The natural logarithm of the response ratio (RR) was used as the effect size of this meta-analysis which can reflect the size of the magnitude of the focused variable in the investigated treatment (NPKM or M) compared to a reference treatment (NPK) (Nony et al., 1995) and calculated by Hedges et al. (1999):

$$RR = \ln(\bar{x}_t/\bar{x}_c) \tag{1}$$

where the subscript of *t* and *c* represents the investigated treatment and the reference treatment, respectively; and \bar{x} is a mean of variable *x*.

The percentages of change in SMBC, SMBN, SMBC/SOC and SMBN/TN from manure relative to NPK were calculated by $(e^{RR} - 1) \times 100\%$ (Luo et al., 2006), where RR_{++} is the weighted response ratio and calculated (Hedges et al., 1999):

$$RR_{++} = \frac{\sum_{i=1}^m \sum_{j=1}^{k_i} w_{ij} RR_{ij}}{\sum_{i=1}^m \sum_{j=1}^{k_i} w_{ij}} \tag{2}$$

where *m* is the number of the level for a given variable, *k_i* is the number of comparisons between manure and mineral fertilizers at the *i*th level, RR_{ij} is the RR for the *i*th level and the *j*th pair, and w_{ij} is the weighting factor for the *i*th level and the *k_i*th pair and expressed as:

$$w_{ij} = \frac{1}{v} \tag{3}$$

where *v* is a variance:

$$v = \frac{SD_t^2}{n_t \bar{x}_t^2} + \frac{SD_c^2}{n_c \bar{x}_c^2} \tag{4}$$

where *n_t* and *n_c* are number of samples in the treatment and the reference, and *SD_t* and *SD_c* are standard deviation of the treatment and the reference, respectively, which are extracted from the publications. If only the standard error (SE) for the treatment and the reference was given in a paper, then *SD* was calculated:

$$SD = SE \sqrt{n} \tag{5}$$

The standard error of RR_{++} was calculated by:

$$S(RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^m \sum_{j=1}^{k_i} w_{ij}}} \tag{6}$$

Therefore, the 95% confidence interval (CI) of (RR_{++}) was given:

$$95\%CI = RR_{++} \pm 1.96S(RR_{++}) \tag{7}$$

If 95% CI for a given focused variable does not overlap with zero, the treatment was considered to represent a significant increase (the overall mean response ratio > 0) or decrease (the overall mean response ratio < 0) compared to NPK (*P* < 0.05). If it overlaps with zero, the treatment was considered to have no significant impact on that variable compared to the reference (*P* > 0.05) (Aloe and Weiss, 2015).

The METAWIN 2.1 software was employed for meta-analysis (Rosenberg et al., 1997). Firstly, we calculated an overall response ratio for all the paired observations (reference and treatment). Then, the ratios were calculated at each level for each categorical variable. Further, between-group heterogeneity (*Q_b*) was examined for a given focused variable (Table 1) to assess the manure effects among the levels of a given variable using the chi-square test. Categorical variable that associated with significant (the value at *P* < 0.05) and large *Q_b* values are considered to have a better ability to predict variation in the overall response ratio relative to other variables in the analysis.

2.3. Statistical analysis

2.3.1. Variance partitioning analysis (VPA)

VPA was used to analyze the contribution of farming practices, soil factors, climatic conditions and their interactions to the focused variables. Farming practices consisted of total C and N input from manure, manure types, practice duration and land use type. Climatic conditions included the mean annual temperature (MAT) and the mean annual precipitation (MAP) for each site, which were collected from the nearest meteorological station (<http://data.cma.cn/>). Soil factors included contents of SOC and TN, total phosphorus (TP), total potassium (TK), available N (AN), available P (AP) and available K (AK), pH, soil clay content, soil bulk density (BD) and soil C/N ratio. Collinearity among these variables was diagnosed by SPSS before VPA analysis (Liang et al., 2016). The analyses were conducted using the vegan package in R program (R version 3.2.2, 2015).

2.3.2. Random Forest model

Over the last two decades the use of RF model has received increasing attention due to the ensemble classification and regression

analysis (Belgiu et al., 2016). This algorithm generates a lot of trees but eventually gives a single prediction with low bias and low variance (Breiman, 2002; Liaw and Wiener, 2002). The RF classifier is composed of combination of tree classifiers where each classifier is generated using random vectors that are independent of the input vector sampling, and each tree votes the most popular projective units to classify the input vectors (Breiman, 2002). The result drawn from the algorithm is considered as more accurate than any of the individual classifiers making up the ensemble (Dietterich, 2002). In our study, we first selected the factors that are significantly related to SMBC and SMBN changes after manure application compared to NPK from the contribution factors that used in VPA analysis by SPSS. Then we employed these dataset (sites) and conducted the RF model to explore the significantly related controlling factors for SMBC and SMBN changes in the cropland in China. There are three important parameters needed for producing forest trees: the number of trees to be generated in the forest (ntree), the number of variables to be selected and tested for the best split when growing the trees (mtry) and the minimal number of observations at the terminal nodes of the trees. We set 1,000 for ntree according to previous published article (Colditz, 2015; Reese et al., 2014) as a higher number will result in more stable estimates of variable importance (Grimm et al., 2008). The mtry was usually the square root of the number of input variables (Gislason et al., 2006). The third parameter was the minimal observation numbers at the terminal nodes of the trees (nodesize) and in our study the value was set to 5 for regression RF. The RF uses the bootstrap repeated sampling method and the out-of-bag data (OOB) as a test sample for RF. The Mean Square Error (MSE_{OOB}) was used to estimate OOB predictions accuracy (Liaw and Wiener, 2002). Differences between observed and predicted value were calculated with the mean percentage error (MPE), root mean square error of prediction (RMSEP), and R^2 to verify the RF model (Liaw and Wiener, 2002; Wiesmeier et al., 2011). The MSE, MPE, RMSEP and R^2 were calculated as follows:

$$MSE = \frac{\sum_{i=1}^n (z_i - \hat{z}_i^{OOB})^2}{n} \quad (8)$$

$$MPE = \frac{\sum_{i=1}^n (pred_i - obs_i)}{n} \quad (9)$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (obs_i - pred_i)^2}{n}} \quad (10)$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (obs_i - \hat{pred}_i)^2}{\sum_{i=1}^n (obs_i - \hat{obs})^2} \quad (11)$$

where \hat{z}_i^{OOB} is the average of all OOB predictions, obs_i is the i th value of the measured dataset, $pred_i$ is the predicted by RF models for the i th value of measured dataset, \hat{obs} is the average of the measured dataset. The RF models were conducted using the "RandomForest" packages (Liaw and Wiener, 2002) in R program (R version 3.2.2, 2015).

3. Results

3.1. Distribution of SMBC, SMBN, SMBC/SOC and SMBN/TN

The normal distributions for content of SMBC and SMBN and ratio of SMBC/SOC and SMBN/TN were shown in Fig. 1 (at $P < 0.001$ level). With manure application, mean contents of SMBC ($400.54 \pm 18.21 \text{ mg kg}^{-1}$; mean \pm 95% CI, thereafter) and SMBN ($61.24 \pm 2.48 \text{ mg kg}^{-1}$) were both significantly higher compared to that in NPK application ($299.92 \pm 13.45 \text{ mg kg}^{-1}$ for SMBC, and $40.99 \pm 1.79 \text{ mg kg}^{-1}$ for SMBN). Meanwhile, manure application significantly increased the mean ratios of SMBC/SOC ($2.77 \pm 0.11\%$) and SMBN/TN ($3.80 \pm 0.14\%$) compared to that in NPK application ($2.52 \pm 0.12\%$ for SMBC/SOC, and $3.34 \pm 0.15\%$ for SMBN/TN).

3.2. Relationship between SMBC, SMBN and manure-C and N

There were significant correlations between SMBC and SMBN for manure and NPK applications (Appendix S2.). The average of SMBC/SMBN ratio was 6.6 for manure application and 7.9 for NPK. SMBC content was positively correlated with annual manure-C input ($R^2 = 0.05$, $n = 197$) and manure-N input ($R^2 = 0.02$, $n = 298$; Appendix S3.). Similarly, SMBN content also had significant correlations with annual manure-C input ($R^2 = 0.12$, $n = 129$) and manure-N input ($R^2 = 0.11$, $n = 222$).

3.3. Variations in SMBC and SMBC/SOC response to manure application

Manure brought an overall 40% increase in SMBC compared to the treatments with NPK application only (Fig. 2). All but two of the ten variables (SOC and soil pH) described in Table 1 significantly affected the variation in the response of SMBC to manure amendment (Table 2). The variable that contributed the most to the variation in this SMBC response was manure type. Application of cattle manure (CT) had the strongest influence on SMBC with a 69% increase, whereas application of farmyard manure (FYM) exerted no significant effect (7.4%) (Fig. 2). Other types of manure had similar effect on SMBC responses (35–43%). The response of SMBC to manure amendment generally increased with increasing experimental duration as well as manure-C and N input. Among all climate types, SMBC was less responsive to manure addition in subtropical monsoon climate (STM; 29%) than in other climate types (45–50%). SMBC in upland cultivated soil was more responsive to manure application (48%) compared to other land-use types (22–33%). The increase in SMBC under manure addition was higher in soils with moderate TN content ($1.50\text{--}2.00 \text{ g kg}^{-1}$) than in soils with low or high TN content. The response of SMBC to manure was comparatively less in soils with clay content $> 30\%$ (32%) than in soils with less clay content (32–50%). Overall manure amendment increased SMBC/SOC by 16% compared to NPK application (Fig. 3). All ten variables significantly affected the variation in SMBC/SOC response to manure amendment (Table 2). Most types of manure that applied increased SMBC/SOC by 18–27%, whereas FYM application resulted in non-significant decrease in SMBC/SOC (-8%). Manure application that lasted for more than 30 years had a significantly larger effect on SMBC/SOC (34%) compared to others with less experimental duration (11–18%). The effect of manure application on SMBC/SOC increased with manure-C and N input, but this increase was significant only when manure-C and N input was greater than $4000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ (50%) and $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (40%) with a minimum of a three-year experimental duration. The largest positive effect of manure application on SMBC/SOC was observed in NTM (25%) among all climate types. The increase in SMBC/SOC by manure was greater where the contents of soil TN and SOC were high. SMBC/SOC was most responsive to manure application in loamy soils (24%) and where soil pH < 8 (18–21%).

3.4. Variations in SMBN and SMBN/TN response to manure application

Across all observations, manure application increased SMBN by 55% compared to application with NPK only (Fig. 4). All but three (climate types, land use types and soil pH) of the 10 variables significantly affected the variation in SMBN response to manure amendment (Table 2). The effect of manure on SMBN was dramatically distinct among different manure types. Application of sheep manure (SP) led to the greatest increase in SMBN by 169%, followed by horse manure (HS; 118%), whereas FYM application resulted in a non-significant decrease in SMBN by 7%. Manure application that lasted for 25–30 years resulted in the greatest increase in SMBN by 85% compared to other experimental duration (40–52%). Similar to SMBC, the response of SMBN to manure amendment increased in consistent with manure-C and N input. The effect of manure application on SMBN was significantly less in soils with high TN content of $> 2 \text{ g kg}^{-1}$ (34%) but

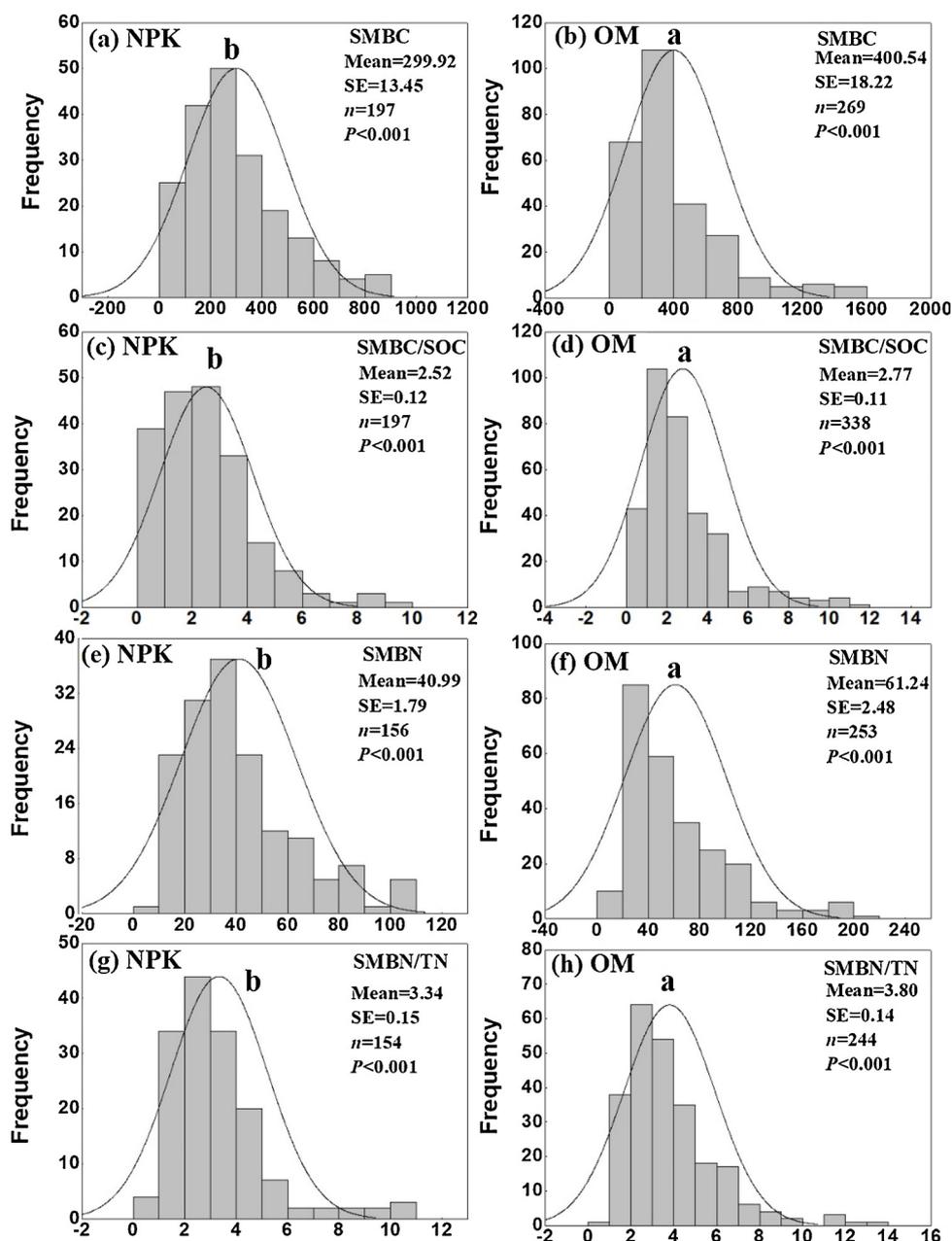


Fig. 1. Normal distribution of SMBC (a and b; mg kg^{-1}), SMBC/SOC (c and d), SMBN (e and f; mg kg^{-1}) and SMBN/TN (g and h). The (a), (c), (e) and (g) represent the NPK treatment, and the (b), (d), (f) and (h) represent the manure treatment. The solid curve is a Gaussian distribution fitted to frequency data. The lowercase a, b above the solid curve represents that significance between different treatments.

greater where SOC content was higher than 12 g kg^{-1} (65–66%). SMBN was less responsive to manure application in clay soils (43%) than in loamy and sandy soils (66% and 68%).

Compared to NPK, manure application provided an overall increase in SMBN/TN by 21% (Fig. 5). Four variables related to manure practice and two of soil variables (TN content and soil texture) significantly affected the variation in the response of SMBN/TN to manure amendment (Table 2). SMBN/TN was significantly increased by application of HS (85%), CT (27%) and swine manure (SW; 19%), while the others had no significant influence. Inputs of manure-C and N input that were greater than $4000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ and $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ resulted in significant increases in SMBN/TN by 108% and 84%, respectively. The increase in SMBN/TN by manure application was the greatest in soil with moderate TN content (39% at $1.5\text{--}2.0 \text{ g kg}^{-1}$) and in loamy soils (39%) among all soil types.

3.5. Controlling Factors of the variations in SMBC and SMBN response to manure application

Results from the variable partitioning analysis showed that 54, 30, 59 and 62% of the variation in the responses of SMBC, SMBC/SOC, SMBN and SMBN/TN, respectively, to manure amendment relative to NPK application could be explained by soil factors, climates, farming practices and their interactions (Fig. 6). Soil factors contributed the most (35%) to the variance in the response of SMBC to manure application, followed by climate (18%) and their interaction (13%), whereas the variation in SMBC/SOC response was mainly explained by climate (29%). The variation in SMBN response to manure can be explained by soil factors (12%), climate (19%), and interactions between soil factors and climate (30%) and between farming practices and soil factors (44%). Soil factors and climate explained 23% and 13% of the variance in SMBN/TN response to manure application, and the interactions of

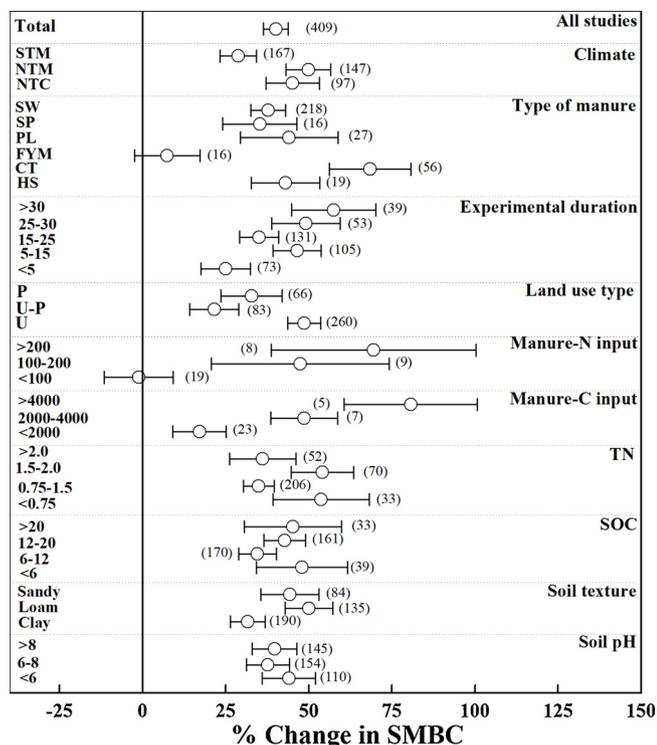


Fig. 2. Percent changes in soil microbial biomass carbon (SMBC) under different categories. Open circles with error bars denote the overall mean response ratio and 95% CI, respectively. The 95% CI that do not go across the zero line mean significant difference between treatment and reference ($P < 0.05$). The value in parentheses represent independent sample size.

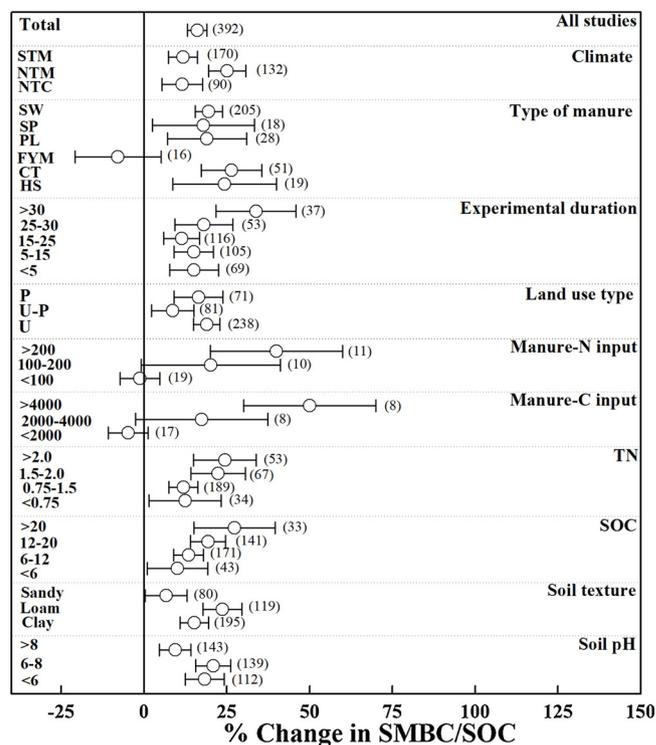


Fig. 3. Percent changes in SMBC/SOC under different categories. Open circles with error bars denote the overall mean response ratio and 95% CI, respectively. The 95% CI that do not go across the zero line mean significant difference between treatment and reference ($P < 0.05$). The value in parentheses represent independent sample size.

Table 2

Between-group variability (Q_b) among observations (n) indicating their potential as predictor variables of SMBC, SMBN, SMBC/SOC and SMBN/TN response to manure application compared to mineral fertilized agricultural fields.

Categorical variables	SMBC		SMBN		SMBC/SOC	
	n	Q_b	n	Q_b	n	Q_b
All studies	410		332		394	
Climate	410	27.10**	332	2.45	392	17.39**
Manure type	352	58.73**	276	62.41**	337	20.88**
Experimental duration	402	34.79**	315	34.15**	380	14.50**
Land use	409	38.20**	326	7.08	390	7.45*
N rate	36	47.99**	28	12.80**	40	9.18*
C rate	35	12.71**	23	37.58**	35	11.20**
TN	361	20.18**	321	22.14**	343	10.24*
SOC	403	6.63	309	23.11**	388	8.90*
Soil texture	409	19.32**	328	18.51**	394	16.05**
Soil pH	409	1.58	323	1.58	394	11.66**

* $P < 0.05$.

** $P < 0.01$.

three categorized factors also accounted for a considerable amount of the variance in SMBN/TN.

The independent variables that used in our RF models overall explained 42% and 32% of the variances in the responses of SMBC (RMSEP = 0.017) and SMBN (RMSEP = 0.029) to manure application relative to NPK application across major croplands in China (Fig. 7a and b). According to the amplitude of increase in MSE, manure-N input, soil TN content and manure were the most important factors affecting the variation in SMBC response (Fig. 7c), whereas the controlling factors for the variation in SMBN response all related to initial soil properties, i.e. available N (AN) and SOC content, and bulk density (Fig. 7d). Besides, the effect of manure-related factors, manure-N input for example, was more significant on variation in SMBC than in SMBN (21% vs. 8%).

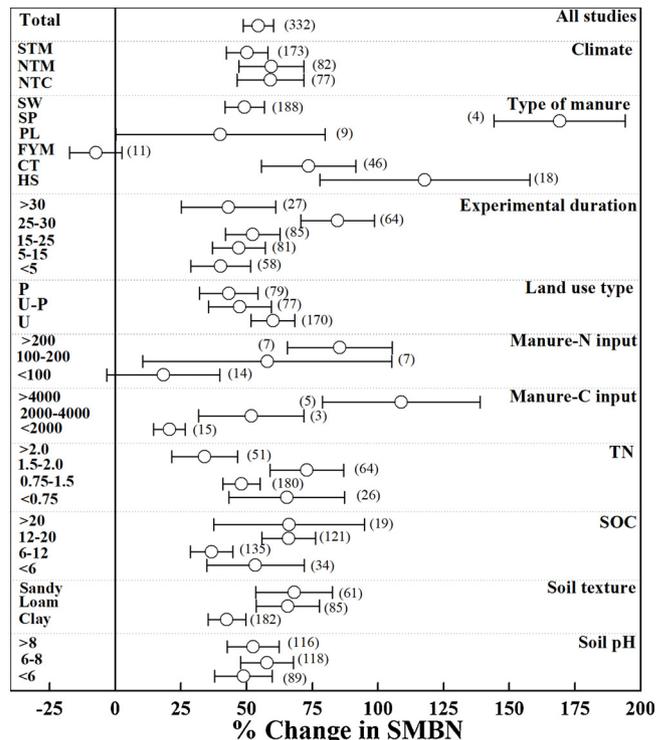


Fig. 4. Percent changes in soil microbial biomass nitrogen (SMBN) under different categories. Open circles with error bars denote the overall mean response ratio and 95% CI, respectively. The 95% CI that do not go across the zero line mean significant difference between treatment and reference ($P < 0.05$). The value in parentheses represent independent sample size.

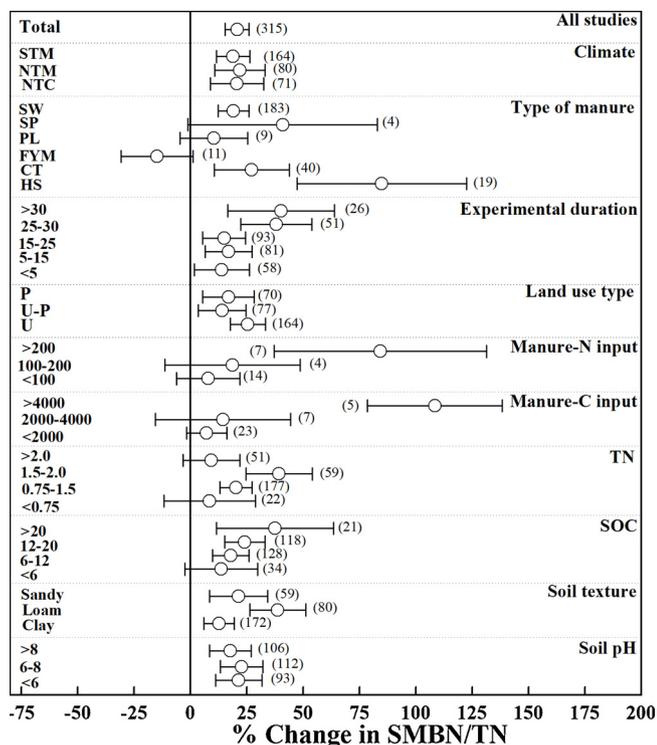


Fig. 5. Percent changes in SMBN/TN under different categories. Open circles with error bars denote the overall mean response ratio and 95% CI, respectively. The 95% CI that do not go across the zero line mean significant difference between treatment and reference ($P < 0.05$). The value in parentheses represent independent sample size.

4. Discussion

4.1. Overall response of soil microbial biomass to manure application

Numerous studies have reported that in agricultural systems application of manure usually resulted in an increase in soil microbial biomass, even though the response of microbial biomass can be highly variable depending on soil types, management practices and climate conditions (Esperschuetz et al., 2007; Lentendu et al., 2014; Linderman and Davis, 2004; Parham et al., 2002; Plaza et al., 2004). In the present study, we analyzed a total of 1448 comparisons between manure and mineral fertilizers and found that manure application overall increased both SMBC and SMBN by 40% and 55%, respectively, across major cultivation systems in China (Figs. 2 and 4). This increase in microbial biomass was higher compared to a globally increase in SMBC by 36% and SMBN by 27% (Kallenbach and Grandy, 2011), suggesting that manure amendment can be more beneficial for microbial communities to recover from long-term and intensively application of mineral fertilizers in conventional agricultural systems of China. Despite the fact that manure is rich in readily available C, N and other macro- and micro-nutrients that microorganisms require for their growth and activities (Feikea et al., 2009; Gupta et al., 1992), there are also various effects of manure that microbes can profit from. For instance, manure application can maintain soil moisture and ease rapid changes in soil temperature (Naeini and Cook, 2000), which helps to provide a stable environment for soil microbes to growth.

Although there was convincingly positive effect of manure application on microbial biomass relative to mineral fertilizer, the mean SMBC of treatments with manure amendment ($400.54 \pm 18.21 \text{ mg C kg}^{-1}$) is still not comparable to that in unmanaged ecosystems, e.g. $670.1 \pm 27.9 \text{ mg C kg}^{-1}$ in grassland soils in China (Zhao et al., 2017). This suggests that other agricultural disturbances such as tillage may restrain the potential recovery in microbial biomass by manure application (Stark et al., 2007). Nonetheless, even a small increase in

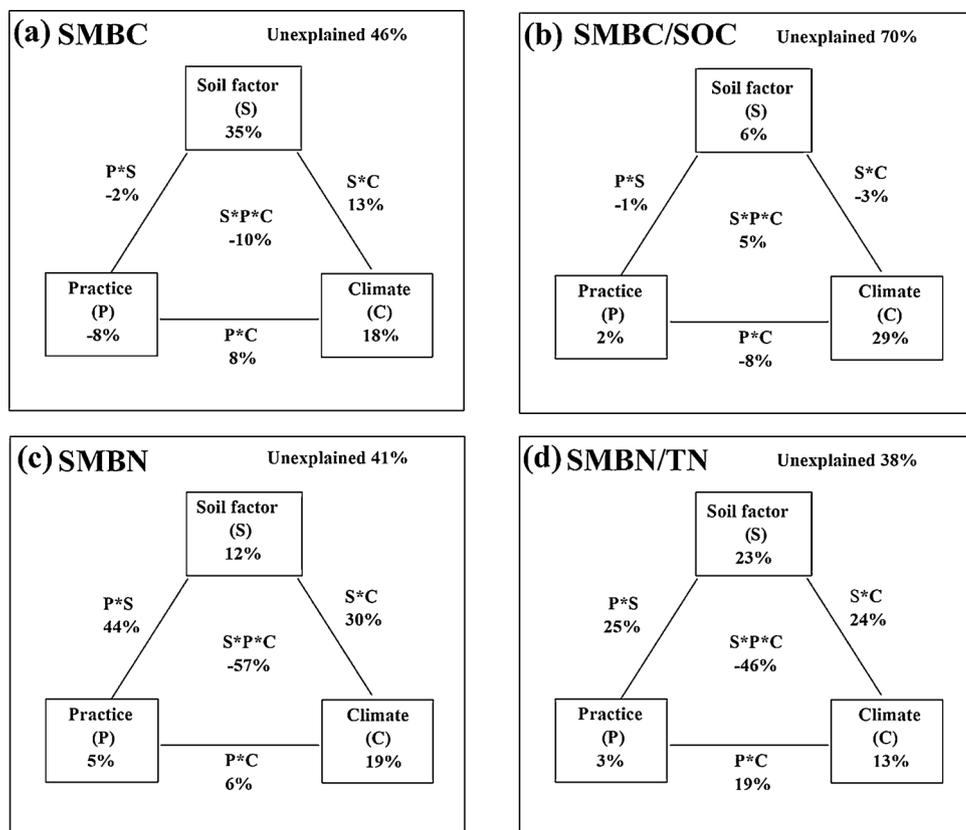


Fig. 6. Variable partitioning analysis (VPA) was used to analyze the effects of soil factors (S), anthropogenic farming practices (P) and climates (C) and their interactions on the variance of SMBC (a), SMBN (b), SMBC/SOC (c) and SMBN/TN (d) in the whole cropland in China for the percentage change among manure and mineral fertilization system.

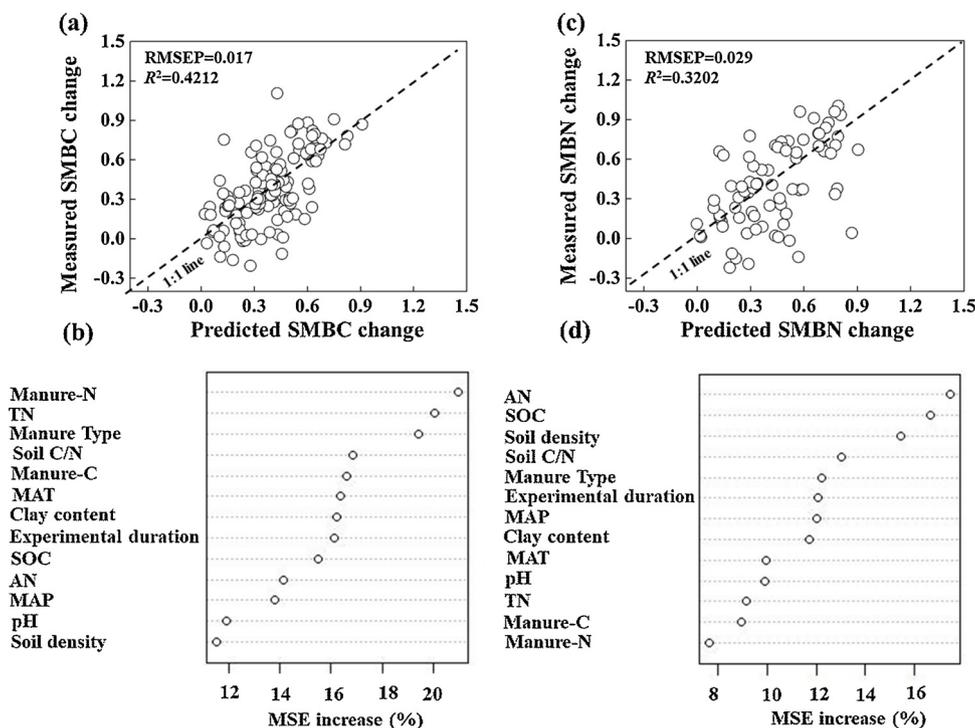


Fig. 7. Relative importance of independent variables for controlling SMBC (a), SMBN (c) changes after manure application as determined using random forests (RF) models and the performance of random forests models for detecting controlling factors of SMBC (b), SMBN (d) change in the croplands in China.

microbial biomass can have significant improvement in belowground C and N cycling as well as associated soil functions. We found that the ratio of SMBC to SOC was significantly increased in treatments with manure application by 16% compared to that with only mineral fertilizers (Fig. 3). SMBC/SOC ratio could reflect the efficiency of the conversion of exogenous C input into microbial biomass C (Anderson and Domsch, 1989; Sparling, 1992) and consequently microbial residues, which are considered as the primary C-containing constituents contributing to the stable SOC pool (Liang et al., 2017). Therefore, application of manure instead of mineral fertilizer alone has a great potential in improving both bio-fertility and C sequestration for major agricultural systems in China.

4.2. Driving factors of microbial biomass responses to manure application

We found that the responses of microbial biomass to manure inputs are highly complex due to variations in factors related to climate condition, management practices and initial soil environment. Results from the VPA analysis suggested that both climate and soil factors imposed considerable degrees of constraint on microbial biomass responses to manure application, whereas the contribution of farming practices did not reach a significant level (Fig. 6). However, manure-related factors were proved crucial for microbial biomass, especially SMBC responses to manure application (Fig. 7). It is possible that the magnitude of the effect of manure-related factors was counteracted by land-use type, which was also included as the farming practice factor but with relatively smaller influence (Table 2). In addition, the effect of farming practices could be overshadowed by soil and climatic factors, e.g. air temperatures and precipitation, due to their primary control on the accumulation of both above- and belowground biomass and hence microbiological processes (Li et al., 2014; Moore and Lobell, 2015), which would consequently constrain the response of microbial biomass to manure application.

4.2.1. Farming practices

Manure-related factors, especially manure type and application rate

(C and N input), imposed the greatest impact on the magnitude of how microbial biomass responses to manure application relative to mineral fertilizer (Table 2; Fig. 7). Application of cattle manure resulted in the greatest increase in SMBC (68%) among all type of manure, which was in consistent with a recent global meta-analysis (Kallenbach and Grandy, 2011). Whereas application of farmyard manure did not lead to significant responses in both SMBC and SMBN as was previously observed (Carpenter-Boggs et al., 2000; Parham et al., 2003). The divergence in the response of microbial biomass to different manure that applied could be closely related to the bio-chemical composition of manure. In general, manure that comprises of larger proportion of low molecular weight C such as sugar and water-soluble C are easier for microbes to utilize than those with high contents of water-insoluble C (e.g. cellulose) or lignin (Valenzuela-Solano and Crohn, 2006). The relative high polyphenols content and C/N ratio in farmyard manure (Ghoshal and Singh, 1995) makes it a low-quality source for microbes to profit from. In addition to C availability, different N forms that manure contained can also lead to the variation in the response of microbial biomass to manure application. For example, contents of NH_4^+ and NO_3^- were typically higher in poultry manure compared to cattle manure (Zhou et al., 2013). Application of poultry manure can therefore result in high NH_4^+ -N concentration in soils, which may cause cyto-toxicity in microbes, suppress enzyme activities, and decrease C use efficiency (Lorenz, 2006; Geisseler and Horwath, 2010). These all could attenuate the positive effect of poultry manure application on soil microbes, leading to a less increase in SMBC compared to cattle manure across China or at a global scale (Kallenbach and Grandy, 2011).

In addition to manure type, the duration of manure application also imposed a significant impact on the variation in the response of microbial biomass (Table 2). We found that the magnitude of positive effect from manure application on SMBC was greatest where the practice lasted for more than 30 years (Fig. 2), which was similar with previous studies that reported the longer the practice lasted, the greater impact manure had on SMBC and SMBN (Bossio et al., 1998; Lundquist et al., 1999). Long-term application of manure can help to rebuild soil

environment that is favorable for microbes by improving soil pH, substantial supply of soil available C and N, and creating well-ventilated conditions beneficial for rapid decomposition of manure and crop residues that will provide energy for the turnover of soil microbes (Ellmer et al., 2015; Haynes and Naidu, 1998; Zhou et al., 2017a).

Land-use type, as a well-known factor influencing microbial community (Jangid et al., 2008), had significant impact on the variation in the response of microbial biomass to manure application (Table 2), yet its impact was relatively weak compared to that of manure-related factors. Our results indicated that SMBC and SMBN are relatively responsive to manure application in upland soils among all land use types. Cropping systems and land management practices can be diverse under different land use types, which could affect the amount and quality of C that returned to soils (Wardle, 1992; Jangid et al., 2011), the decomposition rate of crop residues and manure (Singh et al., 2007), and the utilization of N by microbes (Baijukya et al., 2006; Kushwaha et al., 2000). In addition, land use types can differentially affect edaphic properties such as soil texture, soil C and N availability, pH and microclimate, which can influence physical and metabolic niche diversity in soils (Lauber et al., 2008), thereby resulting in different composition of microbial communities. Besides, manure amendment may enhance soil aggregation in upland soils and thus improve aerobic metabolism (Fenchel and Finlay, 1995), which is more efficient in converting soluble C into microbial biomass compared to anaerobic metabolism (Picek et al., 2000).

4.2.2. Climate types

The response of SMBC to manure application was distinct among different regions of China with varying climatic conditions (Table 2, Fig. 4). Previous studies on geographic distribution of microbial biomass suggested that aboveground productivity contributes a great proportion to the variation in SMBC among different biomes (Wardle, 1992; Fierer et al., 2009). In agricultural systems, however, application of fertilizers and irrigation, as well as controlled crop management, can artificially regulate productivity to similar levels (Kallenbach and Grandy, 2011). Therefore the regional variation in the response of microbial biomass to manure application might be related to direct influence of climate on microbial growth rate instead of plant productivity. Higher temperature and larger amount of precipitation may generally create favorable conditions that are more conducive to the growth of soil microbes. Previous studies also concluded that soil water content was one of the major determinants of soil microbial community composition (Drenovsky et al., 2004). We accordingly anticipated a strong positive response of microbial biomass in region with subtropical monsoon climate (STM), because the higher topsoil temperature and moisture content in STM could increase the availability of C and N and thus improve the growth of microbial biomass (Zhou et al., 2017b). However, our results showed that SMBC was less responsive to manure application in STM compared to higher latitude regions with cooler temperature (Fig. 4). It is possible that under the subtropical cropping systems where climate is not the limiting factor, growth of microbes can be constrained by other factors such as higher turnover rates of both newly entered C and the standing microbial biomass (Zech et al., 1997; Santruckova et al., 2000) and enrichment in iron oxide (Bond-Lamberty and Thomson, 2010; Keener et al., 2000).

4.2.3. Soil factors

We found that the response of microbial biomass to manure application was closely related to the initial levels of SOC, TN and clay content in soils (Table 2; Fig. 7). SOC and TN contents are often considered important indicators of substrate availability and stoichiometry that could directly affect SMBC and SMBN (Li et al., 2015). As such, our results showed that the response of microbial biomass to manure addition was comparatively lower in soils with relatively low SOC concentration. Soils with higher clay content typically have stronger chemical protection on labile C, limiting its accessibility for microbes to

utilize (Six et al., 2000). In accordance, we found that in clay soil the increases in SMBC and SMBN were below the overall mean (Fig. 2 and 4), indicating that the effect of manure application on microbial biomass relative to mineral fertilizers was depressed by high clay content (> 30%) in soil. Previous studies also suggested C sources in newly added manure were better protected from attacking by microorganisms in clay soils than in sandy or loamy soils (Franzuebbers et al., 1996; Gul et al., 2015; Hassink, 1994). In addition, soils with high clay content can have higher exchange capacity, which could lead to a slow decomposition of the added manure (Thomsen et al., 2001) and hence limited effect on the increase of microbial biomass.

Assessing the response of microbial biomass to manure application in major cropping systems across China are difficult because the area is in particular large with intensive human activities. Although we chose the factors that could be more relevant to the variation in SMBC and SMBN responses to manure application, the explanatory power of random forest model was not high. There are many factors that may affect the degree of explanation, such as method of manure stacking and application, farmer's cultivation habits and experiences, as well as sampling time of the studies. In addition, different crop types have distinctive root exudates which can affect the SMBC and SMBN and land-use type with different crop types could best predict shift in microbial community composition (Lauber et al., 2008). Thus, better assessment of the effect of manure application on microbial biomass across major cropping systems of China requires for more detailed studies related to agricultural management method in the future.

5. Conclusion

In the present study, we conducted a meta-analysis to investigate the response of microbial biomass to manure application relative to mineral fertilizers across major cropping systems in China. Our results show that there are substantial increases in SMBC (40%), SMBC/SOC (16%), SMBN (55%) and SMBN/TN (21%) after manure amendment compared to those with mineral fertilizer application. The variation in the response of microbial biomass to manure application is mainly attributed to manure-related factors as well as local climate conditions and inherent soil properties. Among the chosen variables, manure type had the strongest impact on SMBC, with greatest response in systems receiving cattle manure but insignificant response in systems receiving farmyard manure. Soils with relatively low SOC and TN content, and high clay content can constrain the positive effect of manure application on microbial biomass. While there are certain challenges in managing manure input, e.g. maintaining crop yield and reducing environmental risks, implement of manure application can compensate some of the negative effect of intensively fertilized systems by improving soil microbial biomass and associated ecosystem functions.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.still.2019.06.008>.

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