



Soil microbial biomass phosphorus can serve as an index to reflect soil phosphorus fertility

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Abstract

The effect and relative contributions of C and P inputs on soil microbial biomass P (MBP) accumulation were studied in three long-term soil fertility experiments with various soil and climate characteristics at Qiyang, Yangling, and Wulumuqi. The maximum of soil MBP in all three sites was 47.8 mg P kg⁻¹. The MBP accumulated per unit in soil (mg P kg⁻¹ soil) was correlated with a 4.91 mg kg⁻¹ increase in Olsen P. For each unit increase in P surplus (kg P ha⁻¹), manure C (kg C ha⁻¹), and stubble C (kg C ha⁻¹), MBP accumulation increased by 330, 3.7, and 13 units (μg P kg⁻¹ soil), respectively. The soil MBP was positively correlated with crop yield and P uptake, making the soil MBP a useful soil P fertility index. The critical levels of the soil MBP pool were 140 kg ha⁻¹, 57–62 kg ha⁻¹, and 33–35 kg ha⁻¹ in acidic red soil, loessial soil, and grey desert soil, respectively. This is the first report to establish a quantitative index of soil fertility based on the soil MBP pool. Our findings demonstrate that C input is a good driver of soil MBP accumulation. Integration of the soil MBP as an index of soil P fertility into agricultural P management is useful to help manage mineral P fertilizers as part of sustainable agricultural practices.

Keywords Microbial biomass P · Fertility · Yield · Manure · Organic C

Introduction

Phosphorus (P) fertilizer derived from rock phosphate is a non-renewable resource and its availability limits agricultural production worldwide (George et al. 2018). Excessive fertilization leads not only to the rapid depletion of P resources but also results in serious environmental pollution (Almeida et al. 2019; Darch et al. 2018; Li et al. 2019, 2020; Ma et al. 2018). Modern intensive agriculture depends upon inputs of inorganic and organic P fertilizers or a combination of both to ensure high productivity. However, the functioning of agricultural systems also depends on biological processes (Kouno et al. 2002; McLaughlin et al. 2011). In soils, microorganisms control the turnover of P from the decomposition of plant and animal residues and the mobilization of organic phosphates. In addition, mobilized P can subsequently accumulate within the soil microbial biomass itself, the latter being termed microbial biomass P (MBP).

MBP is both a soil P sink and a potentially biologically available P pool for plant and soil microorganisms. On the one hand, soil microorganisms compete with plants for available P (Zhang et al. 2014, 2016). This process decreases P reactions with the soil, e.g., sorption by soil particles,

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precipitation by divalent or trivalent cations, occlusion by iron-aluminum oxides etc. (McLaughlin et al. 2011). On the other hand, the release of P from the soil MBP pool mediates soil P availability (Liebisch et al. 2014; Richardson and Simpson 2011). When soil conditions are changed, e.g., soils undergo drying-rewetting, considerable amounts of P can be released from the soil MBP pool (Turner and Haygarth 2001). The P released from the soil MBP pool occurs as orthophosphate and in organic forms that can be rapidly mineralized in soils (Macklon et al. 1997; Schneider et al. 2017). Previous studies of soil MBP have mainly focused on theoretical aspects, including microbes mediating soil P turnover, mobilization, stoichiometric ratio, etc. However, the soil microbial biomass is widely regarded as an early indicator of changes in soil quality (Bünemann et al. 2018). Although numerous indices have been developed to evaluate soil P fertility based on soil physiochemical properties, relatively few have been developed for MBP.

Due to differences in temperature, moisture, soil matrix, land use type, crop rotations, fertilizers and soil amendments, tillage practices and so on, the soil MBP is highly variable in most intensive agricultural systems. As an active component of the soil P pool, MBP constitutes approximately 1.60% (15.5 mg P kg⁻¹) of the total P pool in intensive agricultural soils (Richardson and Simpson 2011; Xu et al. 2013). The addition of decomposable organic matter, including animal manure, composted organic matter, and straw usually significantly enlarges the soil MBP pool (Ayaga et al. 2006; Ocio and Brookes 1990; Saunders et al. 2012; Singh and Singh 1993). However, previous studies have only focused on one or two aspects of different farming practices, e.g., organic manure, straw, and P fertilizer addition, soil characteristic, or climate region. Thus, there are still critical knowledge deficits regarding the relative contributions of C (stubble C, manure C, and straw C inputs) and P (fertilizer P, manure P, and straw P inputs) to soil MBP accumulation across different soil matrices, land use types, crop rotations, tillage practices, and climate characteristics.

A wide range of studies have indicated that the MBP pool can facilitate a continuous slow release of inorganic P to crops, showing positive relationships between MBP and crop yields (Ayaga et al. 2006; Sugito et al. 2010) as well as crop P uptake (Chen et al. 2000; Saini et al. 2004). However, considering that soil MBP is a highly variable soil P pool in intensive agricultural soils, these results generally relate to individual soil types, crop systems, or greenhouse experiments and cannot provide a general conclusion. The relationships between C inputs, crop P uptake, and the MBP pool in different farming practices, soil types, and climate characteristics are still unclear. However, few studies developed considering MBP increases caused by long-term inputs of different C sources, e.g., stubble C, manure C and straw C, can improve soil P availability and crop P uptake. Achieving a general and reliable

conclusion requires comprehensive and long-term studies. Thus, the next challenge is to empirically test and understand the effect of long-term C inputs on soil MBP accumulation, particularly across a range of soil and climate conditions in China.

To understand the characteristics of soil MBP accumulation and the relationship between MBP pool and crop yield in long-term alternative and conventional farming practices on a large scale, we asked the following questions: (1) What implications might C inputs have regarding the MBP pool and soil P availability? (2) How can crop P uptake and yield be influenced by MBP? We investigated the relationships between different C and P source inputs and the MBP pool and crop yield under six typical fertilization treatments across arid, semiarid, and wet environmental study sites, which were established in 1990. We hypothesized that (i) C inputs can increase the soil MBP pool under different long-term nutrient management practices; (ii) the increased soil MBP pool is related to increased soil P availability; and (iii) crop P uptake and yield increase in association with an enlarged MBP pool.

Materials and methods

Study sites

This study was carried out in three long-term soil fertility experiments at Qiyang, Hunan Province; Yangling, Shaanxi Province; and Wulumuqi, Xinjiang Province, China, which represent arid, semiarid and wet environmental conditions, respectively. These three long-term experiments are part of The National Long-term Monitoring Network of Soil Fertility and Fertilizer Effects, established in 1990. Here, we investigated the relationship between different C and P source inputs and the soil MBP pool to understand the mechanisms of soil MBP accumulation. The experimental locations and period, crop rotations, plot areas, soil types, and climate characteristics are shown in Table 1. Further details regarding soil properties and climate conditions can be found in Zhang et al. (2009), Wang et al. (2015), and Khan et al. (2018).

Experimental design

The experiments consisted of the following six treatments: (1) Control (non-fertilization), (2) NK (combination of N and K fertilizers), (3) NPK (combination of N, P and K fertilizers), (4) NPKS (combination of NPK fertilizers and straw), (5) M (manure), and (6) NPKM (combination of NPK fertilizers and manure). The fertilizer application rates are shown in Table 2. The amounts of N, P, and K added to the plots in manures were estimated based on the N, P, and K concentrations measured in manure applied each year and the mass of manure applied (Isaac and Johnson 1976). All straw or manure was applied once per

Table 1 The experimental location, crop rotations, soil properties, and climate characteristics (Khan et al. 2018; Liu et al. 2010; Tong et al. 2014; Wang et al. 2015; Zhang et al. 2009)

| | Experiment site | | |
|------------------------------------|----------------------|----------------------|-----------------------|
| | Wulumuqi | Yangling | Qiyang |
| Location | 43° 57' N, 87° 46' E | 34° 17' N, 108° 0' E | 26° 45' N, 111° 52' E |
| Precipitation (mm) | 299 | 585 | 1431 |
| Mean annual temperature (°C) | 7 | 13 | 18 |
| Soil classification (FAO) | Haplic Calcisols | Calcaric Regosol | Ferralic Cambisol |
| Organic C (g kg ⁻¹) | 8.8 | 7.4 | 8.6 |
| Total N (g kg ⁻¹) | 0.87 | 0.93 | 1.07 |
| Total P (g kg ⁻¹) | 0.67 | 0.61 | 0.45 |
| Available K (mg kg ⁻¹) | 288 | 191 | 122 |
| Olsen-P (mg kg ⁻¹) | 3.4 | 9.6 | 10.8 |
| Soil pH | 8.1 | 8.6 | 5.7 |
| Crop rotation | Maize–wheat–cotton | Wheat–maize | Wheat–maize |
| Crop rotation cycle | 3 years ^a | 1 year | 1 year |
| Experimental period (year) | 1990–2016 | 1991–2015 | 1991–2015 |
| Plot area (m ²) | 468 | 196 | 196 |

^a The crop rotation cycles for Wulumuqi were maize–spring wheat–winter wheat from 1990 to 2008 and maize–winter wheat–cotton from 2009 to 2016

year in Yangling after harvesting the maize and in Wulumuqi and Qiyang after harvesting each crop. Urea, superphosphate, and potassium chloride were the sources of N, P, and K

respectively in the non-manure treatments. All of the P and K fertilizers and half of the N fertilizers were applied before planting each crop. The remainder of the N was applied as a

Table 2 Fertilizer application rates (kg ha⁻¹ crop⁻¹) in different treatments in three long-term experiments (Zhao et al. 2010)

| Nutrients | Treatments | Wulumuqi ^a | Yangling ^b | | Qiyang ^c | |
|-----------|------------|-----------------------|-----------------------|-------|---------------------|----------|
| | | Maize/wheat/cotton | Wheat | Maize | Wheat | Maize |
| N | NK | 242 | 165 | 188 | 90 | 210 |
| | NPK | 242 | 165 | 188 | 90 | 210 |
| | NPKS | 217 + 29 ^d | 165 + 43 | 188 | 90 + 9 | 210 + 9 |
| | M | na ^e | na | na | 0 + 90 | 0 + 210 |
| | NPKM | 85 + 240 | 115 + 50 | 188 | 27 + 63 | 63 + 147 |
| P | NK | 0 | 0 | 0 | 0 | 0 |
| | NPK | 60 | 58 | 25 | 16 | 37 |
| | NPKS | 51 + 5 | 58 + 4 | 25 | 16 + 1 | 37 + 1 |
| | M | na | na | na | 0 + 13 | 0 + 37 |
| | NPKM | 22 + 65 | 58 + 95 | 25 | 16 + 13 | 37 + 31 |
| K | NK | 51 | 69 | 78 | 30 | 70 |
| | NPK | 51 | 69 | 78 | 30 | 70 |
| | NPKS | 43 + 54 | 69 + 57 | 78 | 30 + 17 | 0 + 135 |
| | M | na | na | na | 0 + 30 | 0 + 135 |
| | NPKM | 10 + 160 | 69 + 180 | 78 | 30 + 30 | 50 + 39 |

^a M = 30.0 t ha⁻¹ sheep manure

^b M = 26.2 t ha⁻¹ cattle manure

^c M = 40.0 t ha⁻¹ pig manure (30% before wheat, 70% to maize)

^d The amount of N/P/K contained in the added fertilizer and crop straw or organic manure

^e not applicable. The amount of N, P, or K inputs are calculated according to the data of N, P, and K concentration ever year

topdressing at the maize male tetrad stage (V12) and cotton bud stages. Weeds were removed by hand during each cropping season. Acetamiprid was applied in the cotton seedling stage (35 days after sowing) to control thrips and thiamethoxam was applied in the cotton bud stage (63 days after sowing) to control aphids in 2015 at Wulumuqi. Phenamacril was applied in the wheat GS65 stage to control wheat scab in 2015 at Yangling (Zadoks et al. 1974). No bactericides or insecticides were applied to the maize at any time.

Sampling and analysis

In Qiyang, soil samples were taken at the maize physiological maturity (R6) growth stage in 2012, maize V12 growth stage and milking stage (R3) in 2015, and maize V12 growth stage in 2018 (Ritchie et al. 1992). In Wulumuqi, soil samples were collected at the cotton bud and boll stage (68 and 103 days after sowing) in 2015 and maize tasselling stage (VT) and R3 growth stages in 2016. In Yangling (without NPKS treatment), soil samples were taken at the wheat GS71 stage and maize VT growth stage in 2015 and 2018. Three randomly collected initial soil samples were made into one composite sample, and a total of three independent replicated samples for each treatment were taken at Qiyang, Yangling, and Wulumuqi (Liu et al. 2010). All samples were taken from the surface horizon (0–20 cm) of each treatment and collected using an auger with an internal diameter of 5 cm. The soil samples were then sieved (< 2 mm) and divided into two subsamples. One subsample was immediately stored at 4 °C until needed for the incubation study, and the other subsample was air-dried and stored for analysis.

The soil organic C (SOC), manure C, and straw C contents were determined through a wet digestion method using potassium dichromate (Kalembasa and Jenkinson 1973). Soil Olsen P was determined by 0.5 M NaHCO₃ extraction (pH = 8.5) according to the Olsen method (Kuo 1996). Soil MBP was measured as described by Brookes et al. (1982). Briefly, three sets of fresh soil equivalent to 5 g oven-dried (24 h, 105 °C) soil were separated into 50 ml soda-glass vials. The first set was left unamended, the second set was spiked with P as KH₂PO₄ equivalent to 25 µg P g⁻¹ oven-dried soil, and the third set was fumigated under vacuum with ethanol-free CHCl₃ (24 h, 25 °C). All three sets of soils were then extracted with 0.5 M NaHCO₃ at a 1:20 soil/reagent ratio and shaken at 150 rev min⁻¹ in an orbital incubator for 30 min (25 °C). P concentrations in the extracts were determined with a molybdenum blue colorimetric method using UV photometry at 880 nm (Kuo 1996). Soil MBP was calculated using a universal conversion factor of 0.4 to account for incomplete extraction of soil MBP (Brookes et al. 1982).

Plant samples (grain and straw) were collected each year at the time of soil sampling. The plant samples were oven-dried, threshed (if required), and weighed, after which they were

ground to pass through a 0.15 mm sieve. The plant and manure total P were measured using the vanado-molybdate method after digestion in a mixture of concentrated H₂SO₄ and H₂O₂ (Thomas et al. 1967).

Statistical analyses

The storage capacity of the soil MBP was calculated using the following equation:

$$\text{soil MBP pool (kg ha}^{-1}\text{)} = c_{\text{mbp}} \text{ (mg kg}^{-1}\text{)} \times d \text{ (cm)} \\ \times \rho \text{ (g cm}^{-3}\text{)} \times a \text{ (ha)} \times 10,$$

where c_{mbp} is the soil MBP, d is the thickness of the surface soil (20 cm), ρ is the bulk density, and a is the soil area.

The manure C input was calculated using the following equation:

$$\text{Manure C input (kg ha}^{-1}\text{)} = M \text{ (kg ha}^{-1}\text{)} \\ \times c_{\text{manure}} \text{ (g kg}^{-1}\text{)} \times 10^{-3},$$

where M is the total manure input and c_{manure} is the C concentration in manure. The straw C input was calculated using the following equation:

$$\text{Straw C input (kg ha}^{-1}\text{)} = S \text{ (kg ha}^{-1}\text{)} \times c_{\text{straw}} \text{ (g kg}^{-1}\text{)} \\ \times 10^{-3},$$

where S is the total straw returned to the soil and c_{straw} is the C concentration in straw. The quantity of stubble was calculated as the sum of root C and extra-root C. On the basis of the results of Barber (1979) and Bolinder et al. (1997, 1999), the assumption that there was a quantity of extra-root C produced equal to the amount of root biomass C at harvest was used in the current study. According to previous studies, the shoot:root ratios that we used for maize, wheat and cotton were 5.0 (Bolinder et al. 1999), 4.9 (Bolinder et al. 1997) and 13 (Xie and Tian 2010), respectively. The stubble C input was calculated using the following equation:

$$\text{stubble C input (kg ha}^{-1}\text{)} = B \text{ (kg ha}^{-1}\text{)} \times c_{\text{biomass}} \text{ (g kg}^{-1}\text{)} \\ \times \text{SR}_{\text{ratio}} \times 2 \times 10^{-3},$$

where B is the total above-ground biomass, c_{biomass} is the weighted average C concentration in above-ground biomass, and SR_{ratio} is the shoot:root ratio of crops. Total organic C input was calculated as the sum of manure C plus straw C plus stubble C.

Manure P input was calculated using the following equation:

$$\text{manure P input (kg ha}^{-1}\text{)} = M \text{ (kg ha}^{-1}\text{)} \times c_{\text{manureP}} \text{ (g kg}^{-1}\text{)} \\ \times 10^{-3},$$

where M is the total manure input and c_{manure} is the P concentration in manure. Straw P input was calculated using the following equation:

$$\text{straw P input (kg ha}^{-1}\text{)} = S \text{ (kg ha}^{-1}\text{)} \times c_{\text{straw}} \text{ (mg kg}^{-1}\text{)} \\ \times 10^{-6},$$

where S is the total straw returned to the soil and c_{straw} is the P concentration in straw. Total P input was calculated as the sum of the manure P plus straw P plus fertilizer P. The P surplus was calculated as the total P input minus shoot P content.

Statistical analyses were performed using the statistical software program SAS ver. 9.4 for Windows (SAS Institute, Cary, NC, USA). All data were checked for homogeneity of variances using the SAS Proc Univariate procedure (Schneider et al. 2017). The residuals were also tested for normality as determined by the Shapiro-Wilk statistic. MBP in different treatments did not respond to either years or crop growth stages. Thus, we combined MBP in different years and growth stages as replicates. ANOVAs were conducted to assess the effect of fertilizer treatments on the soil MBP, DOC, and Olsen P. Soil MBP, DOC, and Olsen P in different treatments and significant differences of means were compared with Duncan's multiple-comparison range test. To compare MBP in two specific fertilization treatments, a paired sample t test was conducted to evaluate the differences. The significance level was set at $P < 0.05$. Regression analysis was used to determine the relationship between SOC, total organic C, stubble C, manure C, straw C, Olsen P, total P input, P surplus, mineral P, manure P, straw P, and soil MBP. To clarify the effects of mineral P fertilizer, manure and straw P, P surplus, SOC, soil dissolved organic C, MBC, manure C and stubble C on soil MBP, and available P, we used structural equation modelling (SEM) to examine the relative importance of soil C input and P input for soil MBP. We evaluated the fit of our model using the χ^2 -test. A linear-plateau model or linear model was used to explore the relationship between crop yields, P uptake, and soil MBP.

Results

MBP in soils under different treatments

The MBP in the NPKM treatments were significantly higher than that in other treatments at all three study sites. The maximum MBP at Qiyang, Yangling, and Wulumuqi was 48.7 mg P kg⁻¹, 56.0 mg P kg⁻¹, and 38.6 mg P kg⁻¹, respectively (mean value 47.8 mg P kg⁻¹) (Fig. 1). However, the mean value of soil MBP at Qiyang, Yangling, and Wulumuqi was 24.1 mg P kg⁻¹, 20.6 mg P kg⁻¹, and 13.6 mg P kg⁻¹, respectively, under intensive management practices (NPK treatment) (Fig. 1). Across the three study sites, the mean MBP increased 143%

in the NPK treatment compared to the NK treatment. Compared to the single application of P fertilizer (NPK) or organic manure (M), soil MBP in the NPKM treatment increased by 89.7% and 17.7%, respectively (Fig. 1), while soil MBP only increased by 8.1% in NPKS treatment compared with that of the NPK treatment (Fig. 1). Soil MBP at the Qiyang and Yangling sites was significantly different in the NPK and NPKS treatments (Fig. 1a, b). However, compared to the NPK treatment, NPK fertilizer and straw (NPKS) addition did not increase the soil MBP at Wulumuqi (Fig. 1c).

Relationships between SOC, organic C inputs and soil MBP

SOC in the Control and NK treatments were significantly lower than that in other treatments at all three study sites. While in the M and NPKM treatments, SOC were significantly increased compared with the other treatments (Fig. 2a). There were positive linear relationships between SOC, total organic C input, stubble C input, manure C input, and soil MBP at all experimental locations (Fig. 3). Specifically, the linear relationships between SOC and soil MBP at Qiyang ($R^2 = 0.91$, $P < 0.01$), Yangling ($R^2 = 0.88$, $P < 0.01$), and Wulumuqi ($R^2 = 0.80$, $P < 0.01$) were significant and positively correlated (Fig. 3a). The linear relationships between soil MBP and C inputs were positive for total organic C input ($R^2 = 0.60$, $P < 0.01$), stubble C input ($R^2 = 0.37$, $P < 0.01$), and manure C input ($R^2 = 0.52$, $P < 0.01$) (Fig. 3b, c, d). There were no positive relationships between straw C input and soil MBP (Fig. 3e).

Relationships between the Olsen P, P surplus, P inputs and soil MBP

The linear relationships between Olsen P and soil MBP at Qiyang ($R^2 = 0.79$, $P < 0.01$), Yangling ($R^2 = 0.86$, $P < 0.01$), and Wulumuqi ($R^2 = 0.86$, $P < 0.01$) were significant and positively correlated (Fig. 4a). A similar positive linear relationship between P surplus and soil MBP was also found ($R^2 = 0.68$, $P < 0.01$, Fig. 4c). When the P surplus increased by 1 kg P ha⁻¹, the MBP increased by 330 $\mu\text{g P kg}^{-1}$ soil (Fig. 4c). The linear relationships between total P inputs and soil MBP were positive and significant for total P input ($R^2 = 0.64$, $P < 0.01$), mineral P input ($R^2 = 0.14$, $P = 0.02$), and manure P input ($R^2 = 0.43$, $P < 0.01$) (Fig. 4b, d, e). However, there was no significant relationship between straw P input and soil MBP (Fig. 4f).

Relationships between crop yield, P uptake and the soil MBP pool

There were positive linear relationship between soil MBP and crop P uptake in Qiyang (Fig. 5a). However, P uptake of

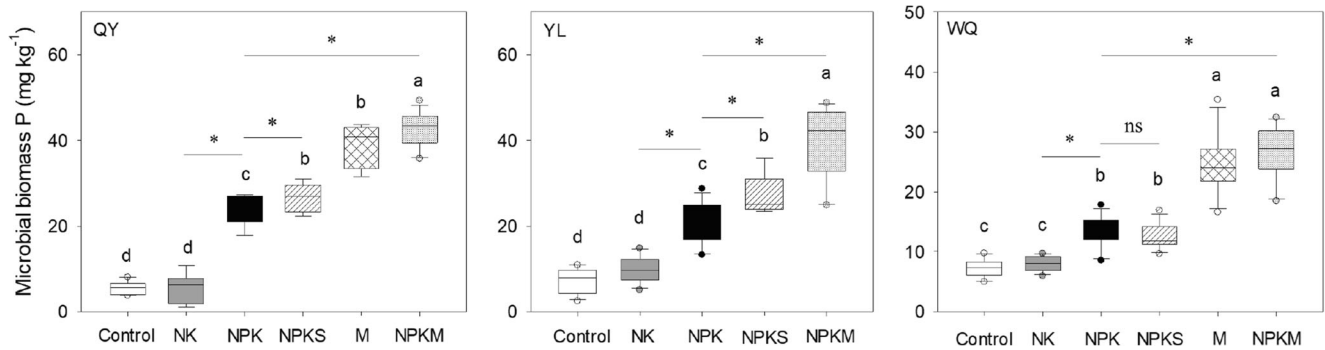


Fig. 1 Microbial biomass P in Control (non-fertilization), NK (N and K fertilizers combination), NPK (N, P, and K fertilizers combination), NPKS (NPK fertilizers and straw combination), M (manure), and NPKM (combination of NPK fertilizers and manure) treatments at the

Qiyang (QY), Yangling (YL), and Wulumuqi (WQ). Different letters indicate significant differences in MBP between treatments ($P = 0.05$). The * indicates significant differences between two specific fertilization treatments (paired sample t test, $P = 0.05$)

wheat, maize, and cotton in Yangling or Wulumuqi showed a linear-plus-plateau model with soil MBP (Fig. 5b–e). In Qiyang, the NPKM treatment had the highest maize grain yield in 2012, 2015, and 2018, while Control, NK, and NPK treatments had relatively lower maize grain yields. There was a significant positive relationship between soil MBP and maize grain yield ($R^2 = 0.82$, $P < 0.01$, Fig. 5f). In Yangling, the wheat grain yields were higher in the NPK, NPKS, and NPKM treatments than in the NK and Control treatments in 2015 and 2018, and a similar trend occurred for summer maize during the same years. Wheat and maize yields were positively correlated with soil MBP up to thresholds (the point at which yield did not increase with an increase in soil MBP) of $57.0 \text{ kg P ha}^{-1}$ ($R^2 = 0.82$, $P < 0.01$, Fig. 5g) and $62.3 \text{ kg P ha}^{-1}$ ($R^2 = 0.75$, $P < 0.01$, Fig. 5h), respectively. The yields of cotton and maize were higher in the NPK, NPKS, M, and NPKM treatments than the NK and Control treatments at Wulumuqi in 2015 and 2016. Cotton and maize

yields were positively correlated with soil MBP up to thresholds of $32.9 \text{ kg P ha}^{-1}$ ($R^2 = 0.84$, $P < 0.01$, Fig. 5i) and $35.1 \text{ kg P ha}^{-1}$ ($R^2 = 0.53$, $P < 0.01$, Fig. 5j), respectively, beyond which the cotton and maize yields did not increase with soil MBP.

The effects and relative importance of C and P inputs on MBP

We generated a structural equation model based on the known effect and relationship between key drivers of soil MBP accumulation and soil P availability (Fig. 6). This model explained 23% and 33% of the variance in the soil MBP and P availability, respectively. P surplus, manure C, and stubble C are the most important predictors of soil MBP accumulation (Fig. 6a, b). We also found that MBP had positive effects on soil P availability, and soil MBP and P surplus were the most important predictors of soil P availability (Fig. 6a, c).

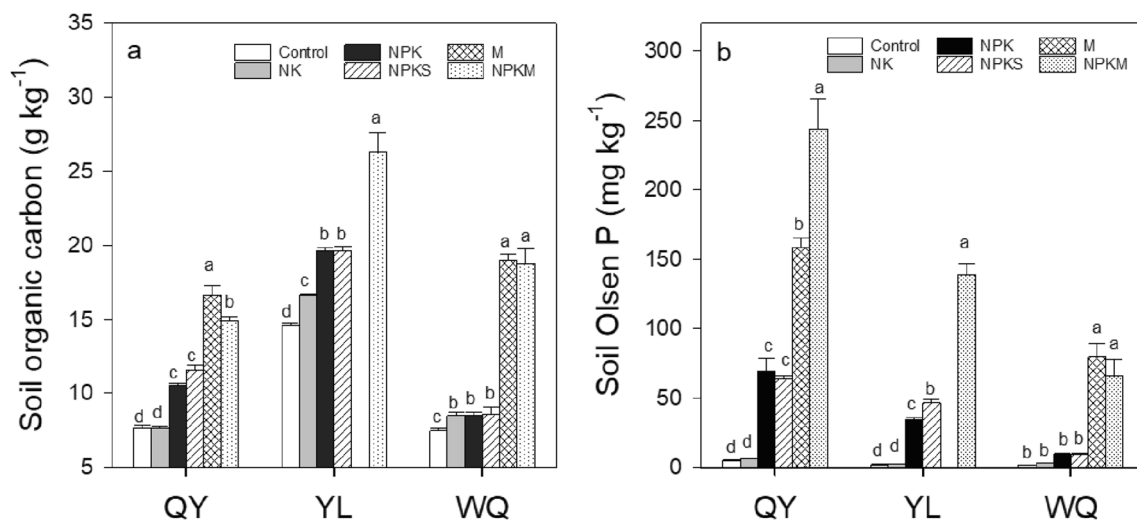


Fig. 2 SOC concentration (a) and soil Olsen P (b) in different fertilizer treatments. QY (Qiyang), YL (Yangling), and WQ (Wulumuqi). Different letters indicate significant differences in SOC or Olsen P between different treatments ($P < 0.05$)

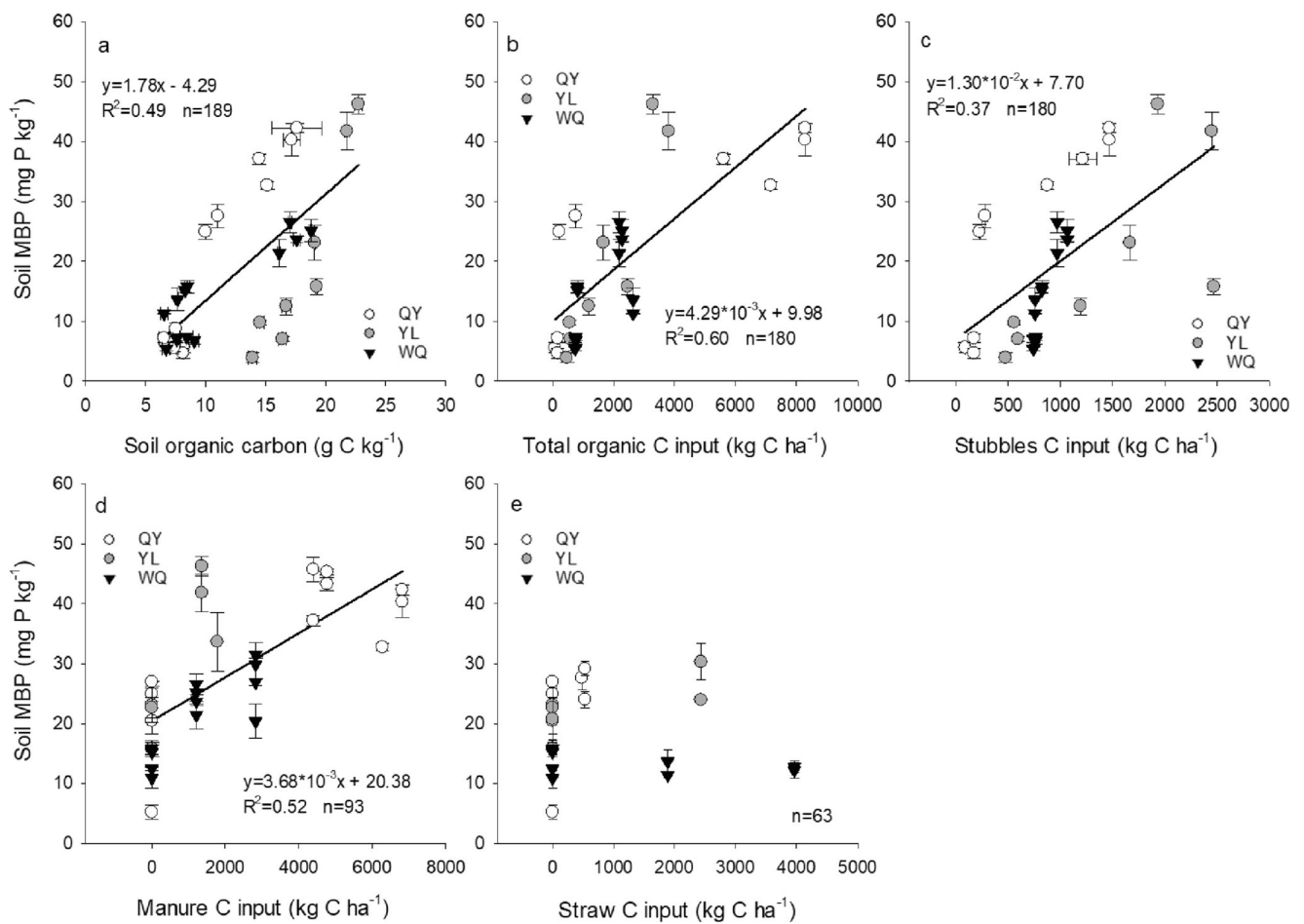


Fig. 3 Relationships between soil organic C, total organic C input, stubble C input, manure C input, and straw C input against soil MBP in three sites. Different study sites are represented by different shapes of data

points and represent Qiyang (QY) (white dots), Yangling (YL) (grey dots), and Wulumuqi (WQ) (black triangle)

Abundance index of soil P fertility

Soil fertility also depends on capability of microbes to enhance crop productivity by increasing the availability of P. According to the relationships between soil MBP and crop yield, we propose MBP to be a soil microbiological indicator for soil P fertility (Fig. 5 and Table 3). Based on the yield potential response to MBP, soil P fertility could be divided into 5 calibration levels, i.e., very low, low, medium, high, and very high. These categories represent less than 70%, 70% to 80%, 80–95% and more than 95% of relative yield, respectively, against the MBP test. Critical level refers to the soil MBP that is related to 95% relative yield. For the Qiyang acid soil, the critical level of soil MBP for maize was 140 kg ha⁻¹. For the Yangling loessial soil, the critical levels of soil MBP for wheat and maize were 57 kg ha⁻¹ and 62 kg ha⁻¹, respectively. The critical levels of soil MBP for cotton and maize were 33 kg ha⁻¹ and 35 kg ha⁻¹ respectively in the Wulumuqi grey desert soil, (Table 3). Compared to the NPK treatment, the combination of manure and mineral P fertilizer additions (NPKM treatment) significantly improved crop P uptake and

yield without extra mineral P fertilizer inputs (Fig. 5, Table 2). The mean average partial productivity of P fertilizer (PFP) values were 57.2 kg kg⁻¹ and 91.3 kg kg⁻¹ under low (NPK treatment) and high (NPKM treatment) soil P fertility levels respectively (Fig. 7).

Discussion

The capacity of the MBP pool in intensive farmland

High soil MBP is strongly related to high soil P availability (Liebisch et al. 2014; Richardson and Simpson 2011). Although microorganisms effectively uptake available orthophosphate from the soil solution (Bünemann et al. 2004; Oehl et al. 2001), P can also be released from the MBP pool due to microbial turnover (Schneider et al. 2017). Our results suggest that MBP had a significantly positive linear relationship with soil available P (Fig. 4a). The maximum value of MBP (47.8 mg P kg⁻¹) was much higher than those of the average intensive cropland soils (15.5 mg P kg⁻¹) and similar to those

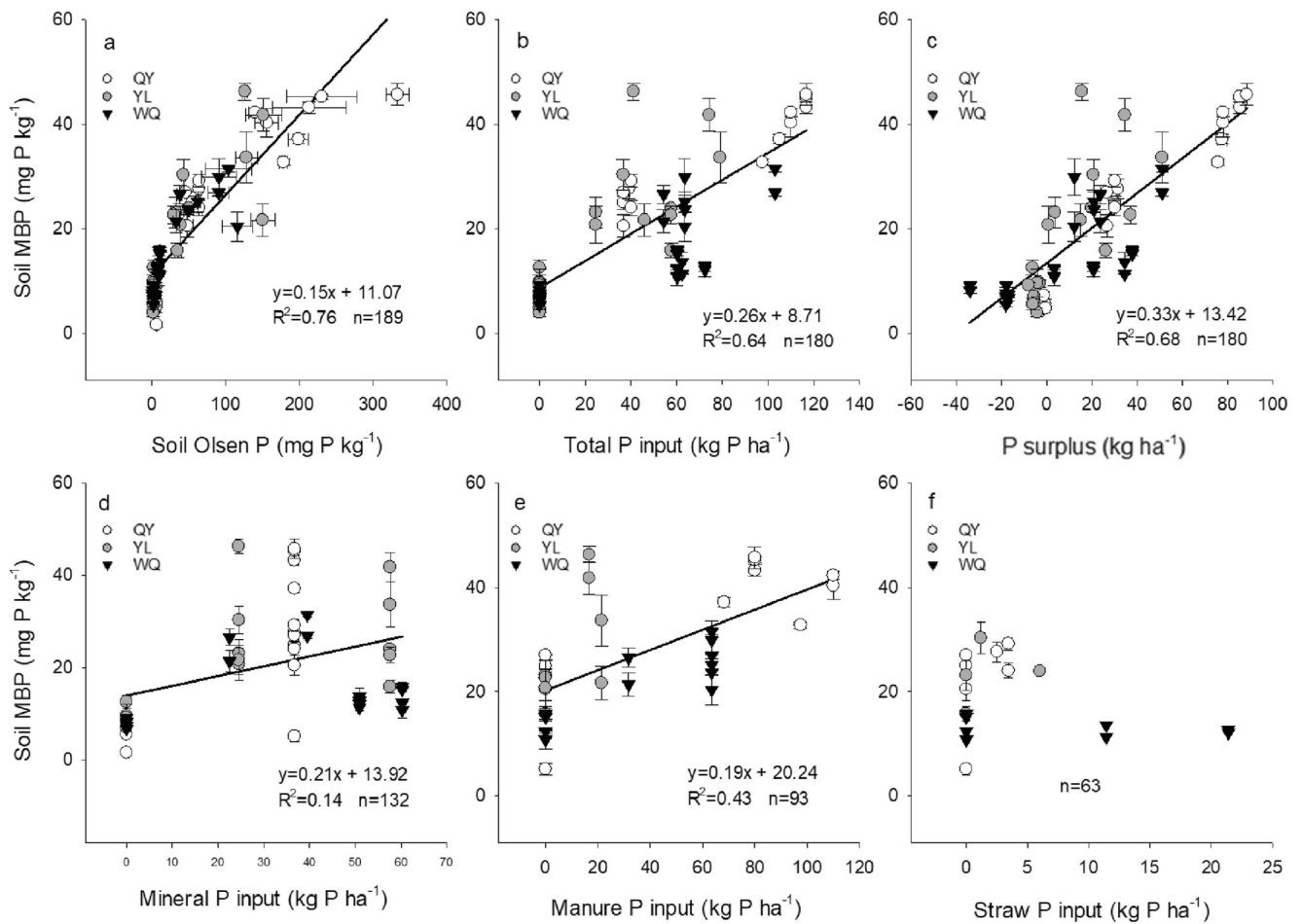


Fig. 4 Relationships between MBP against Olsen P, total P input, P surplus, mineral P input, and manure P input, respectively. Different study sites are represented by different colors and shapes of data points and represent Qiyang (QY) (white dots), Yangling (YL) (grey dots), and Wulumuqi (WQ) (black triangle)

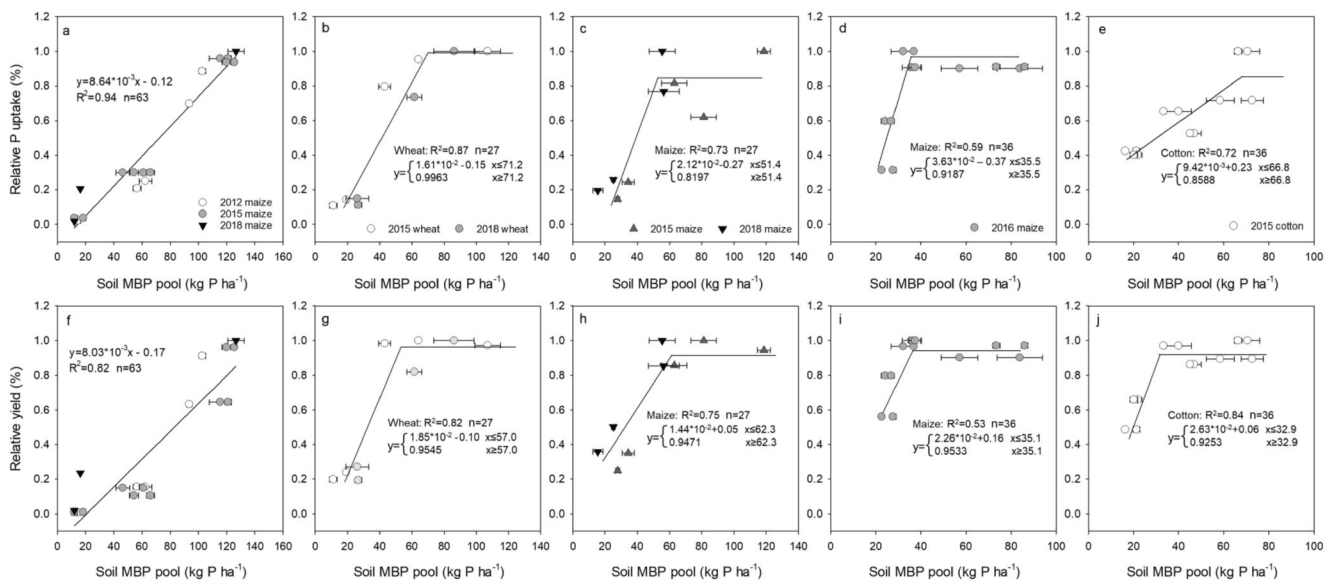


Fig. 5 Relationships between crop P uptake, yield against MBP pool in Qiyang (QY) (panel a, f), Yangling (YL) (wheat: panel b, g; maize: panel c, h) and Wulumuqi (WQ) (maize: panel d, i; cotton: panel e, j) across all treatments. Solid lines are linear regression lines of the piecewise regression or linear regression models. Different crop seasons are represented by different shapes of data points

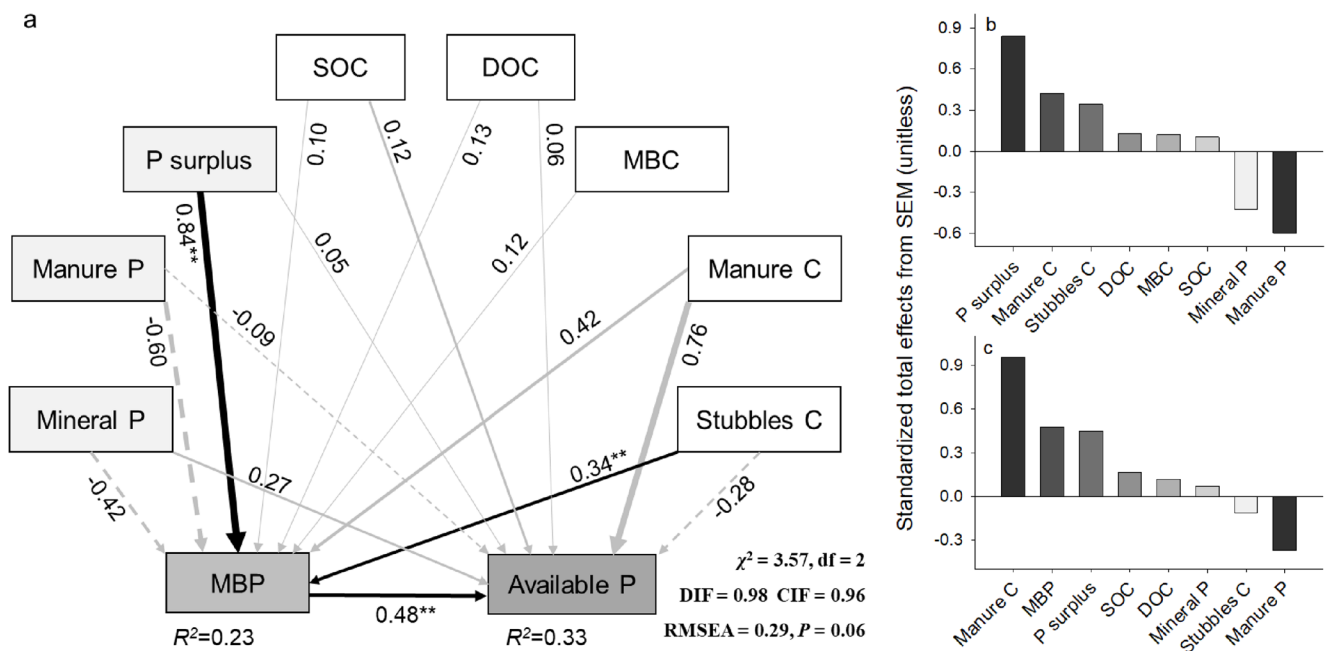


Fig. 6 a Effects of C and P on the soil MBP and available P. Numbers adjacent to arrows are standardized path coefficients and indicative of the effect size of the relationship. The proportion of variance explained (R^2) appears alongside every response variable in the model. Goodness-of-fit

statistics for the model are shown in the lower right corner. ** $P < 0.05$. b Standardized total effects on MBP, which were derived from structural equation modelling. c Standardized total effects on available P, which were derived from structural equation modelling

of forest (58.6 mg P kg⁻¹) and grassland (43.4–69.2 mg P kg⁻¹) soils (Cleveland and Liptzin 2007; Xu et al. 2013). Thus, there is still great potential to increase the MBP pool in intensive farmland soils (e.g., NPK treatment). The enhancement of soil fertility and intensive agricultural management are not mutually exclusive, and both can be combined by increasing the soil MBP.

Impact of different inputs on soil MBP

There was a strong relationship between stubble C (root exudates and root biomass C) and soil MBP (Fig. 3c), where increases in stubble C stimulated increases in the soil MBP without exogenous C and P inputs. When stubble C increased by 1 kg C ha⁻¹, the MBP increased by 13.0 μg P kg⁻¹ soil (Fig.

3c). MBP accumulation can be driven by microbial C acquisition from which plants potentially can benefit, and in turn, plants may provide more C through rhizosphere deposition input to soil microorganisms (Spohn and Kuzyakov 2013). Our findings support that below-ground C allocation increases soil C availability in the rhizosphere, which stimulates microbial growth and thus increases the soil MBP pool (Dennis et al. 2010; Paterson 2003; Tang et al. 2014).

Manure C played important roles in the increase of soil MBP (Figs. 1, 3d, and 6). When manure C increased by 1 kg C ha⁻¹, the MBP increased by 3.7 μg P kg⁻¹ soil (Fig. 3d). This may be because the number of soil microorganisms can be increased by additions of manure alone. Considering that soil microorganisms also need P to maintain their growth, the organic C in the manure stimulated microbial growth (microbial biomass C increase) and

Table 3 MBP pool as an index of soil P fertility

| Relative yield | | < 70% | 70–80% | 80–90% | 90–95% | > 95% | |
|---|------------------|----------|--------|---------|---------|-----------|-------|
| Abundant degree | | Very low | Low | Middle | High | Very high | |
| MBP pool ^a (kg P ha ⁻¹) | Acid red soil | Maize | < 108 | 108–121 | 121–133 | 133–140 | > 140 |
| | Loessial soil | Wheat | < 43 | 43–49 | 49–54 | 54–57 | > 57 |
| | | Maize | < 45 | 45–52 | 52–59 | 59–62 | > 62 |
| | Grey desert soil | Cotton | < 24 | 24–28 | 28–32 | 32–33 | > 33 |
| | | Maize | < 24 | 24–28 | 28–33 | 33–35 | > 35 |

^a The soil MBP pool was calculated using the following equation: soil MBP pool (kg ha⁻¹) = c_{mbp} (mg kg⁻¹) × d (cm) × ρ (g cm⁻³) × a (ha) × 10, where c_{mbp} is the soil MBP, d is the thickness of the surface soil (20 cm), ρ is the bulk density and a is the soil area

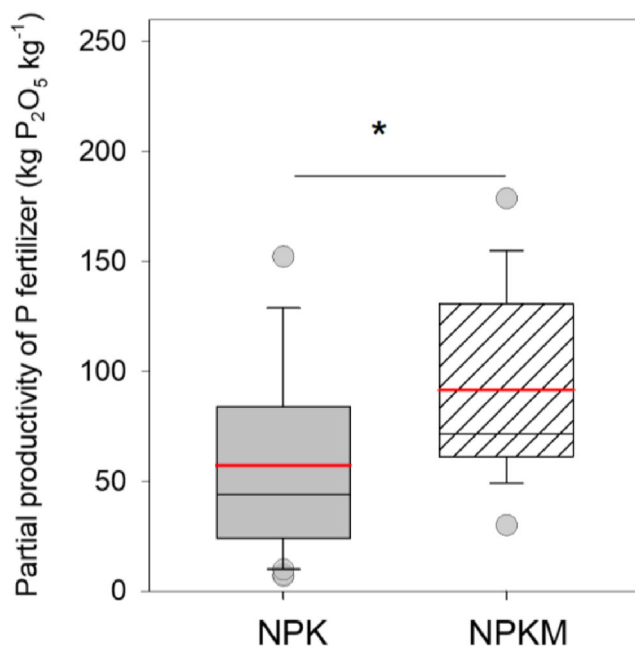


Fig. 7 Partial productivity of P fertilizer in NK and NPK treatments. Abbreviations in the figure: NPK (N, P, and K fertilizers combination), and NPKM (combination of NPK fertilizers and manure). The * indicates significant differences between two fertilization treatments (paired sample t test, $P = 0.05$)

this increased the soil MBP (Cleveland and Liptzin 2007). However, glucose inputs can also increase the soil MBP highlighting the important role of C sources in managing the soil MBP (Kouno et al. 2002; Spohn and Widdig 2017). However, we did not find a positive relationship between straw C input and soil MBP (Fig. 3e). This is likely because the manure contains more dissolved organic C, which is much more readily available to microbes than in straw. Furthermore, straw has a higher C/P ratio than manure, and high substrate C/P ratio will restrict microbial biomass formation due to P limitation (Khan and Joergensen 2019).

Phosphorus input is another significant factor in affecting the MBP pool (Fig. 1). These results were in agreement with previous studies showing that mineral P fertilizer input results in significant increases in soil MBP (Huang et al. 2016; Liu et al. 2012; Shi et al. 2013; Turner and Wright 2014). However, the low R^2 in the relationship between mineral P input and soil MBP indicated that sole inorganic P input was not main factor in MBP accumulation (Fig. 4d). We showed that the P surplus was the key driver for stimulating increases in soil MBP (Fig. 6), because not only soil P is known to be an important driver of soil MBP pool (Roberts et al. 2013; Yang et al. 2010), but also because soil microorganisms have shown luxury absorption of P, which can be immobilized in live microbial cells and their associated pool of metabolites (e.g., poly-P) (Bünemann et al. 2008; Bardgett and van der Putten 2014; Richardson et al. 2009; van der Heijden et al. 2008). The significant positive effects of P input on soil MBP were

mainly due to the enhanced growth of crops and accumulation of soil organic C through increased below-ground C allocation (Zhong and Cai 2007). This study also found that manure P was positively related to soil MBP (Fig. 4d), but there was no significant relationship between straw P input and soil MBP (Fig. 4e). On the one hand, straw with a high C/P ratio may have resulted in limited availability of P, which inhibited the increase in soil MBP (Kouno et al. 2002). On the other hand, straw had less C availability than manure, but the microbial synthesis of organic and immobilization of P was only enhanced by the addition of P in the presence of available C (Bünemann et al. 2008).

The combination of manure and phosphate fertilizer additions improved soil P and C availability, stimulating the maximum soil MBP accumulation (Figs. 1 and 2), confirming previous studies showing that the co-application of mineral P with goat manure produced up to twofold more soil MBP than either mineral P or goat manure applied alone (Gichangi et al. 2009). In addition, a several-fold increase in the soil MBP in response to added C occurred in both P-amended and P-limited soils (Bünemann et al. 2004). This was likely because synthesis of microbial P was enhanced by P addition in the presence of more available C. Significant amounts of pyro- or polyphosphate were likely produced by microorganisms when the availability of P was very high (Bünemann et al. 2008).

Soil MBP pool as an indicator of P fertility was related to crop P uptake and yield

Soil enzyme activity, microbial community composition and activity, and microbial biomass C, N, and P are all important indices of soil fertility (Ren et al. 2019). Most current studies on soil P fertility have focused on phosphatase activity, the qualitative and quantitative analysis of bacteria harbouring genes, which encode these phosphatases, such as alkaline phosphatase (*alp*), quinoprotein glucose dehydrogenase (*gdh*), or β -propeller phytase (*bpp*) (Dai et al. 2020; Zhang et al. 2020), and microbial biomass C:N:P stoichiometry (Wei et al. 2020). Some studies have tried to establish the relationship between these indices and soil P fertility; however, they did not directly develop a quantitative index of soil fertility for P.

In our study, there were positive relationships between the soil MBP pool and crop P uptake (Fig. 5). Although there was probably competition for P between plants and microbes, increases in the soil MBP could have still stimulated plant P uptake (Malik et al. 2013). The MBP pool is an important source of available P in soils even under conditions of high Olsen P (Sugito et al. 2010). Firstly, the soil MBP pool may provide a continuous slow release of inorganic P, which plants can use more efficiently than a single large pulse of P fertilizer (Achat et al. 2012; Ayaga et al. 2006; Bünemann et al. 2013; Turner and Haygarth 2001). During the MBP turnover, plants may be able to take up orthophosphate before its adsorption to

soil colloids, precipitation with Fe, Al, and Ca or occlusion by iron-aluminum oxides, especially in soils with moderate to large P-sorption capacities, as observed for the acidic soil in Qiyang (Bünemann et al. 2004; Ehlers et al. 2010). Secondly, a high MBP pool is associated with the rapid MBP turnover (Brookes 2001). Generally, MBP turnover has been shown to be similar to the MBP value, and more rapid than the corresponding plant P uptake (Liebisch et al. 2014).

The improvement in soil P availability increased MBP pool (Fig. 4a). Crops were significantly correlated with soil MBP (Ayaga et al. 2006; Sugito et al. 2010). Our results in the acidic Qiyang soil are consistent with previous studies whereas there was a linear-plus-plateau relationship between the soil MBP pool and crop yields in the alkaline Yangling and Wulumuqi soils (Fig. 5). Urea application to the intensive cropping systems in the acidic Qiyang soil might have caused further soil acidification (Cai et al. 2015). Likely, soil acidification significantly limited soil P availability and limited crop yields in the NPK treatment, and thus, there was not a linear-plus-plateau relationship between soil MBP and crop yield in the acidic Qiyang soil (Fig. 5f).

We propose MBP to be a microbiological indicator of soil P fertility (Fig. 5 and Table 1). The PFP values for the NPK and NPKM treatments further support that MBP can be used as an indicator of soil P fertility and significantly indicates the mineral P fertility. However, the MBP pool was affected by soil moisture and temperature (Blackwell et al. 2010), competition between plants and microorganisms for P (Rousk et al. 2007), and inputs of mineral P fertilizer, organic manure, straw and plant residues under field conditions (Chen et al. 2003). Thus, the MBP can change during the growing crop season. On the one hand, the fluctuations in MBP mean that microbial P turnover occurs during the growing crop season, and plant-available P can be released from soil microbiome, especially from acido-calcisomes or vacuoles in saprotrophic fungi and in arbuscular mycorrhizal fungi. However, the reported ranges in MBP fluctuations during the cropping seasons are contradictory because they ranged from 80 to 130 mg P kg⁻¹ with relatively high temporal resolution by Perrott et al. (1990), whereas smaller fluctuations in soil MBP (73 to 93 mg P kg⁻¹) were reported by Perrott et al. (1992). Although MBP was dynamic over time, MBP values in the different crop seasons were similar in each treatment in this study. Thus, the mean average soil MBP across different crop growth stages may be a useful index for fertilization recommendations. This study is the first report establishing a quantitative indicator of soil fertility by using MBP. This may contribute to agricultural sustainability by carefully using P resources (Bender et al. 2016).

Conclusion

The P surplus, manure C, and stubble C were key factors affecting soil MBP. When the P surplus, manure C, and

stubble C increased by 1 kg ha⁻¹, the MBP increased by 330, 3.7, and 13 µg P kg⁻¹ in red soil, loessial soil, and grey soil, respectively. Soil MBP had a positive linear relationship with soil available P. The slope of this linear regression was 4.91. Furthermore, the soil MBP was positively correlated with crop yield and P uptake in both acidic and alkaline soils. The critical levels of the MBP were 140 kg ha⁻¹, 57–62 kg ha⁻¹, and 33–35 kg ha⁻¹ in the acidic red soil, loessial soil, and grey desert soil, respectively. Thus, the MBP pool and the microbial P dynamics should be considered for an efficient P use so as to optimize crop yield.

Author contribution Gu Feng and Minggang Xu designed the study. Yisheng Duan, Weige Huo, Xueyun Yang, Xihe Wang, and Boren Wang performed the sampling and analysis. Yi Peng and Gu Feng prepared data set and performed statistical analyses. Yi Peng, Gu Feng, Martin S.A. Blackwell, and Minggang Xu contributed to statistical interpretation of results and wrote the manuscript. Gu Feng contributed to revise the manuscript and provided financial support. All authors read and approved the final manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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