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Towards a conversion factor for soil microbial phosphorus

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ABSTRACT

The available methods of microbial phosphorus (P) analysis do not allow full cell lysis during fumigation and complete P extraction. Consequently, a correction of microbial P (P_{mic}) extraction efficiency (Kp factor) is always necessary. Here we evaluated possible under- or overestimation of microbial P by comparing the direct determination of Kp for various soils with the Kp values obtained from the literature.

We determined a soil-specific Kp value for Cambisol by coupling ^{33}P labeling, anion exchange membranes (AEM) water extraction and liquid fumigation with $CHCl_3$. The measured Kp for the Cambisol (0.69) was much higher than the Kp commonly used in the literature over the last 35 years (0.4). Experimentally measured Kp avoided overestimation of microbial P by more than 1.7 times. Therefore, a soil-specific Kp correction of extractability is a prerequisite for microbial P analysis.

To improve P_{mic} accuracy without direct Kp determination, we conducted stepwise regression analysis between soil-specific Kp values from the literature and soil parameters. The Kp increased linearly with decreasing total P. An exponential increase of Kp with decreasing organic C (R^2 = 0.45–0.76) revealed a threshold of 10 g C_{org} kg^{-1}. Combining three soil parameters in multiple regression – Kp = 0.76–0.007*C_{org} - 0.56*P_{tot} + 0.004*Clay – enables an excellent Kp prediction (R^2 = 0.99).

We conclude that the Kp value of 0.4 commonly used for estimating microbial biomass P cannot be accepted as a constant. Thus, in the absence of soil-specific Kp, we recommend using the regression models considering the basic soil properties.

1. Introduction

The soil microbial P pool (P_{mic}) plays a very important role in P cycling and is highly dynamic. Microbial P serves as both a P source for plants via turnover and mineralization of organic P, and as a P stock by immobilizing inorganic labile P. P_{mic} changes significantly in response to environmental alterations, e.g. drying-rewetting and freezing-thawing [1]. Overall, the soil P cycle is mainly controlled by microbial activity [2]. Globally, P_{mic} averages 8% of the total P, increasing from 1.6% in agricultural soils (high total P, but low microbial biomass) to 15% in soils of shrub ecosystems [3]. In forest soils, however, the P_{mic} can exceed 25% of total P and reaches about 80% of the organic P pool [4]. This calls for reliable P_{mic} measurements to evaluate the P cycle, especially in forest soils.

To date, no standardized methods of P_{mic} analysis – such as those for microbial C and N [5] – are available. Consequently, the global storage and local importance of P_{mic} has not been estimated yet due to the absence of sufficient and reliable data [3]. The determination of P_{mic} mostly follows the protocols using either gaseous chloroform fumigation [6,7] or liquid fumigation in the presence of anion-exchange resin membranes [8]. Both these approaches of P_{mic} determination, however, do not ensure: 1) full cell lysis, 2) complete extraction of microbial cell components from soil, and 3) the determination of the portion of phosphate released from microbial cells but precipitated on sesquioxides, with Ca^{2+} and Mg^{2+} or absorbed by organic matter [9–11]. Therefore, a substantial portion of microbial P bound in the cell materials remains non-extracted and will not be analyzed. This calls for a factor to convert extracted P to total microbial biomass P for correct P_{mic} estimation in soil [12].

For the last 35 years the conversion factor (Kp) 0.4 proposed by Brookes et al. [6] has been commonly used as a constant value. To date, the Kp factors were estimated either 1) based on commercially available lyophilised cells [6], or 2) by adding fungal and bacterial cultures to soil suspensions [13,14] using ^{32}P as a tracer of added P. These Kp's

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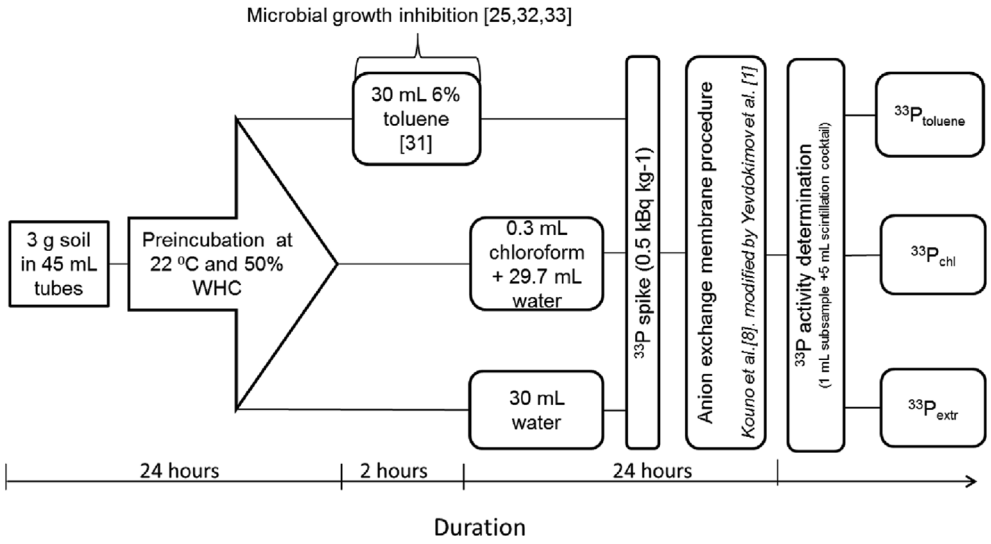

Fig. 1. Schema of the method for soil-specific Kp determination with ^{33}P technique.

cannot adequately reflect extractability because the extraction of the added microbial cells does not necessarily correspond to that of microorganisms developed in soil and attached to surfaces of clays and organic matter. Furthermore, such Kp's differ for methods of P extraction [15] and between the soils. This is because the structure of microbial communities varies greatly depending on pH, $CaCO_3$, sesquioxide content and other soil properties [16]. Moreover, soil microorganisms can rapidly change their physiological state in adapting to altered conditions such as drying, wetting and freezing [17]. This, in turn, affects microbial P extractability. Kp is therefore likely to vary in response to increased substrate, nutrients and altered soil C/N/P ratios [12] because of the different extraction efficiencies of immobilized P. The result is erroneous estimates of P_{mic}.

Usually, the Kp factor is not estimated in each experiment or for individual soils: it is either taken from the literature [18–20] or is not used at all [21–23]. Importantly, disregarding the extraction efficiency results in 5–50% underestimations of P_{mic} [1]. Applying the commonly used Kp 0.4 also rarely gives an accurate P_{mic} content. It causes both under- [12] or overestimation of microbial P. Tests of potential Kp values designed to find a reliable Kp factor yielded contrasting conclusions: either 0.45 [24] or 1 [25] was the most probable estimate.

Inaccurate Kp determination affects not only the estimations of microbial P pools, but also of the related P fluxes, e.g. P immobilization in microorganisms and P mineralization rates. For example, underestimating microbial P immobilization resulted in overestimation of net P mineralization [25]. In contrast, overestimation of P immobilization due to the use of a non-soil specific Kp value may result in confusingly low or even negative values of net P-mineralization [26].

The best estimate of P_{mic} requires determining Kp for specific soil conditions [16] or at least for soil types with similar properties. It is also important to arrive at a common method that is appropriate for many soils in order to obtain comparable data.

In-situ ^{32}P or ^{33}P labeling techniques have been used to verify conversion factors [2,27]. Such techniques are complicated due to isotopic exchange between labeled and non-labeled P pools. This requires an additional conversion factor to account for this isotopic exchange [28]. Estimation of both ^{33}P sorption (R_{sorp}) and ^{31}P–^{33}P isotopic exchange (R_{exch}) during the liquid chloroform fumigation procedure in the range of soils with contrasting properties demonstrated a reasonably stable value of 0.9 for both coefficients [1].

Application of Anion Exchange Membranes (AEM) with the liquid fumigation method [8] strongly increased the recovery of added P [29], enabling accurate *in situ* ^{33}P tracing. Furthermore, the effects of texture and clay content on the P extraction efficiency were less pronounced for AEM combined with liquid fumigation than for gaseous fumigation-extraction [29].

Our study aimed: 1) to determine experimentally the Kp factor for a low P forest Cambisol, as this soil type is missing in the range of the soils tested even though Cambisols are among the dominating soils worldwide; 2) to calculate the relative over- or underestimation by using the Kp from the literature or no Kp vs. the soil-specific Kp estimated for Cambisol and other parameters available in the literature; 3) to detect the relationship between under- or overestimated P_{mic} and P cycle parameters via simple sensitivity analysis; and 4) to prove the mechanistic relationships between soil quality indices and specific Kp's for several soil types to use them in future studies for Kp prediction and P_{mic} calculation.

2. Materials and methods

2.1. Soil-specific Kp determination for Cambisol

Soil was sampled (0–20 cm depth) at the site Unterluess (LUE) located in the district Celle of Lower Saxony (Germany) in the Lueneburg Heath (52°50.32′N, 10°16.06′E, 115 m a.s.l.). The mean annual rainfall is 779 mm and the mean annual temperature is 8 °C. The soil type is a Hyperdystric Folic Cambisol developed from Pleistocene sediments. Further details on the site and soil profile can be found under [29,30].

This soil is strongly P limited and contains the least amount of total, available and microbial P among all soils in the Priority Program *Ecosystem Nutrition: Forest Strategies for Limited Phosphorus Resources* (SPP1685) [29]. Soil pH was 3.7; C_{tot}, N_{tot} and P_{tot} were 43.0, 1.7 and 0.14 g kg^{-1}, respectively. The 0–20 cm layer contained 780, 170 and 50 g kg^{-1} of sand, silt and clay, respectively [29].

The soil-specific Kp was determined by ^{33}P labeling (Fig. 1). Before the experiment, 3 g of air-dried equivalent soil was pre-incubated in 45 mL tubes at 22 °C and 50% WHC until obtaining constant CO_2 emission rates. To establish the ^{33}P added baseline due to both physicochemical processes (sorption and isotopic exchange) during 1 d incubation, a 6-% water suspension of toluene [31] was added to the control treatment as a microbial growth inhibitor (soil-toluene solution ratio was 1:10) [25,32,33]. Then the soil suspension was pre-incubated for 2 h before adding the ^{33}P spike.

Chloroform (0.3 mL) and deionized water (29.7 mL) were added to the soil to account for ^{33}P fixation by sorption and isotopic exchange during chloroform fumigation. Only deionized water (30 mL) was added for the determination of extractable ^{33}P ($^{33}P_{extr}$).

The ^{33}P spike addition (0.1 mg P kg^{-1} soil as KH_2PO_4, 0.5 kBq g^{-1}