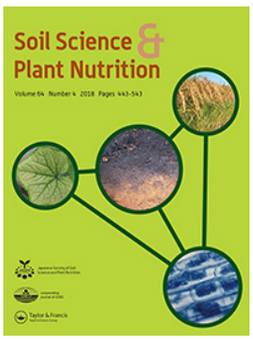

Soil Science and Plant Nutrition

ISSN: 0038-0768 (Print) 1747-0765 (Online) Journal homepage: https://www.tandfonline.com/loi/tssp20

Conversion factor (*k* factor) for estimation of soil microbial biomass potassium by the chloroform-fumigation extraction method

Kohei Yamashita, Hiroki Honjo, Makoto Shinohara & Susumu Asakawa

To cite this article: Kohei Yamashita, Hiroki Honjo, Makoto Shinohara & Susumu Asakawa (2018) Conversion factor (*k* factor) for estimation of soil microbial biomass potassium by the chloroform-fumigation extraction method , Soil Science and Plant Nutrition, 64:4, 465-468, DOI: 10.1080/00380768.2018.1469953

To link to this article: https://doi.org/10.1080/00380768.2018.1469953

Published online: 03 May 2018.

SOIL SCIENCE AND PLANT NUTRITION
2018, VOL. 64, NO. 4, 465–468
https://doi.org/10.1080/00380768.2018.1469953

ARTICLE

OPEN ACCESS Check for updates

Conversion factor (*k* factor) for estimation of soil microbial biomass potassium by the chloroform-fumigation extraction method

Kohei Yamashita[a*], Hiroki Honjo[b†], Makoto Shinohara[c] and Susumu Asakawa[a]

[a]Soil Biology and Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi, Japan; [b]Crop Institute, Aichi Agricultural Research Center, Anjo, Aichi, Japan; [c]NARO Institute of Vegetable and Tea Sciences, Tsu, Mie, Japan

ABSTRACT

The conversion factor, k_K, for estimation of microbial biomass potassium (K) by the chloroform-fumigation extraction method was determined for some arable soils: upland field soils under different fertilization conditions, an upland field soil under a greenhouse condition, and a paddy field soil under a flooded condition. The k_K value varied with land utilization (paddy or upland) or fertilization (chemical or organic fertilizer). Value of k_K was different between paddy field soil (0.28–0.38) and upland field soil (0.41–0.73). This study indicates that the value could be useful for the estimation of microbial biomass K in soil by the chloroform-fumigation extraction method and further investigation of the amounts of biomass K in different types of soils under conditions with varied field managements will be necessary.

ARTICLE HISTORY
Received 22 December 2017
Accepted 24 April 2018

KEY WORDS
Chloroform-fumigation extraction method; conversion factor (*k* factor); paddy and upland fields; soil microbial biomass potassium

1. Introduction

Amounts of soil microbial biomass potassium (K) were recently determined in some upland field soils (Lorenz *et al*. 2010) and paddy field soils (Yamashita *et al*. 2014) by the chloroform-fumigation extraction method that was established by Lorenz *et al*. (2010). It was shown that microbial biomass K could be an important K source for plants (Lorenz *et al*. 2010; Yamashita *et al*. 2014). The conversion factor (k_K factor) is requisite for calculating the biomass K from the flush K ([K extracted in fumigated soil] − [K extracted in non-fumigated soil]) by the chloroform-fumigation method. Values of the k_K factor have been reported for upland field soil (0.21) by Lorenz *et al*. (2010) and for paddy field soil (0.26) by Yamashita *et al*. (2014). Lorenz *et al*. (2010) determined values of k_K factor for three kinds of cornfield soils in Ohio with different textures – fine sand, silt loam, and clay, which ranged from 0.18 to 0.24. They proposed an average value (0.21) as the k_K factor to estimate biomass K, but also suggested that the value could be used until further studies have been conducted in different soil ecosystem and the value should not be generalized. Yamashita *et al*. (2014) determined the value of k_K factor for a paddy field soil in Japan under a drained condition later. These values are the sole measurements of k_K factor so far that were obtained from a limited range of soils. Further estimations are still needed in various agricultural soils under different conditions as repeatedly mentioned in the studies by Lorenz *et al*. (2010) and Yamashita *et al*. (2014).

In this study, we estimated k_K factor for some arable soils including upland field soils under different fertilization conditions, an upland field soil under a greenhouse condition and a paddy field soil under a flooded condition. The objective of this study is to accumulate information about the factor and strengthen the assay method for microbial biomass K.

2. Materials and methods

2.1. Soil samples

One experimental paddy field located at Crop Institute of Aichi Agricultural Research Center, Anjo, Aichi, Japan (Anjo; 34°58′N, 137°4′E) and two experimental upland fields located at the Nagoya University Farm, Togo, Aichi, Japan (Togo; 35°6′N, 137°4′E) and the NARO Institute of Vegetable and Tea Sciences, Ano, Tsu, Mie, Japan (Ano; 34°76′N, 136°4′E) were used (Table 1). A soil sample was taken from the paddy field under a flooded condition with cropping of rice (*Oryza sativa* L.) on 14 August 2013. The paddy field plot was 500 m^2 with mono-cropping of rice. The upland field in Togo was a field with long-term fertilizer treatments and the field experiment has been continued since 1987 (Katayama *et al*. 1998; Islam and Toyota 2004). Two plots were used: a plot with chemical fertilizer (CF – 500 kg N ha^{-1} y^{-1}, 133 kg P_2O_5 ha^{-1} y^{-1}, 500 kg K_2O ha^{-1} y^{-1}) and a plot with chemical fertilizer (500 kg N ha^{-1} y^{-1}, 133 kg P_2O_5 ha^{-1} y^{-1}, 500 kg K_2O ha^{-1} y^{-1}) and 40 t ha^{-1} y^{-1} of farmyard manure (CM). The field had double cropping in a year and the cultivation history has been described by Mitsuboshi *et al*. (2018). Since 1998 sweet corn (*Zea mays* L.) in spring and Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) in autumn have been cultivated. Each plot was 100 m^2 and the soil samples were collected on 14 August 2013 from the bare field after

CONTACT Susumu Asakawa asakawa@agr.nagoya-u.ac.jp Soil Biology and Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

*Present address: Katakura & Co-op Agri Corporation, Chiyoda, Tokyo 102-0073, Japan.
†Present address: Higashi Mikawa Agriculture, Forestry, and Fisheries Office, Imure, Toyohashi 440-0833, Japan.

Table 1. Soils used in this study.

Soil	Crop	Soil type	Soil texture	Total C (g kg^{-1})	Total N (g kg^{-1})	pH (H_2O)	Ex-K[a] (mg K kg^{-1})
Paddy field soil (Anjo)[b]	Rice (*Oryza sativa* L.)	Oxyaquic Dystrudept	LiC	14	1.2	6.2	165 (49[c])
Upland field soil (Togo; CF plot)	Maize (*Zea mays* L.)	Typic Dystrudept	LiC	9.5	1.2	5.3	177
Upland field soil (Togo; CM plot)	Maize (*Zea mays* L.)	Typic Dystrudept	LiC	29	3.0	5.6	415
Upland field soil (Ano; greenhouse)	Ginger (*Zingiber officinale* Roscoe)	Typic Melanudand	SiCL	72	3.6	6.1	ND[d]

[a] Exchangeable potassium; [b] data from Yamashita *et al*. (2014); [c] data under a flooded condition in this study; [d] not determined.
CF, chemical fertilizer; CM, chemical fertilizer with farmyard manure.

harvesting sweet corn. The upland field (150 m^2) in Ano was managed under greenhouse conditions and the soil sampling was conducted on 7 January 2014 from the bare field after harvesting ginger (*Zingiber officinale* Roscoe). The greenhouse had been managed annually under cropping with tomato (*Solanum lycopersicum* L.) or cucumber (*Cucumis sativus* L.) from spring to summer before the sampling. Chemical fertilizers (200 kg N ha^{-1}, 200 kg P_2O_5 ha^{-1}, 200 kg K_2O ha^{-1}, 440 kg CaO ha^{-1}, 310 kg MgO ha^{-1}) and cattle manure compost (10 t ha^{-1}) were annually applied to soil before cultivation of the crop. Four soil samples (about 500 g and located >2 m apart) were collected from each plot at the depth of 0–10 cm of plow layer, combined into a polyethylene bag, and transported to the laboratory. The soil samples were then passed through a 2-mm mesh sieve and stored at 4°C until use.

2.2. Determination of *k* factor

The k_K factor was determined according to the method described by Castellano and Dick (1991) and Lorenz *et al*. (2010); procedures for preparation of mixed bacterial or fungal cultures from soil suspension, inoculation of the microbial suspensions to soil samples, and chloroform-fumigation extraction of the inoculated and uninoculated soil samples were already reported in the previous study (Yamashita *et al*. 2014).

Briefly, 10 g of the soil sample were diluted with 95 mL of 0.15 M NaCl solution supplemented with two drops of Tween 20. One mL of the 10^{-4} diluted suspension was inoculated to 750 mL nutrient broth (8 g L^{-1}) with 100 mg L^{-1} cycloheximide for growing bacteria or 1 mL of the 10^{-1} diluted suspension to 750 mL potato dextrose broth (24 g L^{-1}) with 100 mg L^{-1} streptomycin chloride and 5 mg L^{-1} tetracycline hydrochloride for growing fungi. The preparations were incubated at 25°C for 4 days. One mL of the culture solution was spread onto the respective solid medium supplemented with 15 g L^{-1} agar and incubated at 25°C for 4 days. Lawn of microorganisms grown on the plate was harvested and suspended in 1 mL of 0.15 M NaCl solution by homogenization with a vortex mixer (VORTEX-GENE 2; M&S Instruments Inc., Osaka, Japan). The suspension was washed with 1 mL of 0.15 M NaCl solution three times by centrifugation and decantation to remove free K outside the cells. One mL of the mixed bacterial or fungal suspension was inoculated to 10 g fresh soil sample and mixed. K was extracted and determined in the inoculated and uninoculated soil samples after chloroform-fumigation by the chloroform-fumigation extraction method (Vance *et al*. 1987; Lorenz *et al*. 2010; Yamashita *et al*. 2014). Non-fumigated soil was used as a control. The submerged soil sample was spread in a Petri dish to form a thin soil layer according to Inubushi *et al*. (1984) for the chloroform-fumigation. The 10 g portion of the fumigated or non-fumigated soil was extracted with 50 mL of 1 M ammonium acetate by shaking at about 200 spm and 25°C for 30 min for determination of the biomass K and exchangeable K, where the ratio of soil to extractant and the shaking condition were 1:10 and 200 rpm for 20 min, respectively, in the original literature by Lorenz *et al*. (2010). Inorganic K in the extracts was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (IRIS model; Nippon Jarrell-Ash Co., Ltd., Kyoto, Japan) at 766.4 nm (Yamashita *et al*. 2014). Total K in the bacterial and fungal suspensions was determined by the boiling HNO_3 method according to Helmke and Sparks (1996); the suspension (1 mL) in 0.15 M NaCl was boiled with 10 mL of 1.0 M HNO_3 solution for 15 min and filtrated with a filter paper (Advantec No.6; Toyo Roshi Kaisha Ltd., Tokyo, Japan) and a 0.45 µm pore filter unit (DISMIC®-25CS; Toyo Roshi). Inorganic K in the filtrate was determined by the ICP-AES (IRIS model; Nippon Jarrell-Ash).

Ratios of bacterial biomass to fungal biomass, B:F, were determined according to the substrate-induced respiration (Anderson and Domsch 1973) and selective inhibition (Anderson and Domsch 1975) methods described by Bailey *et al*. (2008). Concentrations of glucose, streptomycin, and cycloheximide were optimized to be 0.5 mg g^{-1} soil, 8 mg g^{-1} soil, and 8 mg g^{-1} soil, respectively, based on preliminary experiments as recommended by Bailey *et al*. (2008). Respired CO_2 was determined by a gas chromatograph equipped with TCD (Shimadzu GC-14B, Kyoto, Japan). Bacterial (k_{Kb}) or fungal (k_{Kf}) factor was estimated as [(K in fumigated soil with bacterial or fungal inoculation) − (K in fumigated soil without bacterial or fungal inoculation)]/(K in the bacterial or fungal suspension) and the k_K factor was calculated as $(B/B + F) \times k_{Kb} + (F/B + F) \times k_{Kf}$ as described by Yamashita *et al*. (2014). The determination was performed in triplicate.

Tukey test was performed using the PAST program (Hammer *et al*. 2001).

3. Results and discussion

The flushes of K after chloroform-fumigation of the soils inoculated with mixed cultures of bacteria and fungi are shown in Table 2,

Table 2. Extracted K after chloroform-fumigation for paddy and upland soils inoculated with bacterial or fungal suspension with known K concentration.

	K concentration (mg K kg^{-1} soil)			
Soil with treatment	Microbial suspension	Inoculated (a)	Uninoculated (b)	(a)–(b)
1. Paddy field soil (Anjo; flooded)				
1 + bacteria	59.8^{CD}	74.5	46.7	27.8^{BC}
1 + fungi	84.3^{E}	74.8	46.3	28.6^{C}
2. Paddy field soil (Anjo; drained)[a]				
2 + bacteria	43.8^{ABC}	189	173	16.1^{A}
2 + fungi	88.2^{E}	193	173	20.2^{ABC}
3. Upland field soil (Togo; CF plot)				
3 + bacteria	71.0^{D}	180	158	22.5^{ABC}
3 + fungi	40.4^{ABC}	187	167	19.3^{AB}
4. Upland field soil (Togo; CM plot)				
4 + bacteria	53.0^{BCD}	416	391	24.7^{ABC}
4 + fungi	31.6^{AB}	410	388	16.6^{A}
5. Upland field soil (Ano; greenhouse)				
5 + bacteria	34.8^{AB}	594	569	22.1^{ABC}
5 + fungi	25.0^{A}	574	553	20.3^{ABC}

[a] Data from Yamashita *et al*. (2014).
CF, chemical fertilizer; CM, chemical fertilizer with farmyard manure.
Different letters (A, B, C, D, E) indicate a significant difference ($P < 0.05$, Tukey test).

which were used for the calculation of the factor. K concentration in the microbial suspension inoculated into soil was significantly higher ($P < 0.05$, Tukey test) for fungi than bacteria in paddy field soils and for bacteria than fungi in CF plot soil of Togo field. However, the other two upland field soils in CM plot of Togo and Ano showed no significance between bacteria and fungi though K concentration tended to be higher in bacteria than fungi as observed in the CF plot. The differences probably reflected the variations in the microbial community structures in the respective soils because the mixed bacterial and fungal cultures were cultivated from the soils with antibiotics. Lorenz *et al*. (2010) reported higher K concentration in fungal suspension than in bacterial suspension that were cultivated from a cornfield soil. However, the increase in the flush K, i.e., (K in fumigated soil with bacterial or fungal inoculation) − (K in fumigated soil without bacterial or fungal inoculation), after chloroform-fumigation was not significantly different between the soils inoculated with bacterial suspension and those with fungal suspension. Lorenz *et al*. (2010) also found that the increase in the flush K by the inoculation of microbial suspension was not significantly different between the soils with bacterial suspension and fungal suspension, irrespective of K concentrations in the microbial suspensions.

Table 3 shows the k_K factor with the B:F ratios in the soils. The recalculated value of k_K factor was shown in Table 3 for the paddy field soil under a drained condition using data for the k_{Kb} and k_{Kf} factors in the previous study (Yamashita *et al*. 2014). The B:F ratio was not significantly different (Tukey test) among the soils and ranged from 35:65 to 44:56, indicating that fungal biomass dominated in the soils and the ratio was slightly higher than the values (10:90 to 35:65) for agricultural soils reported by Anderson and Domsch (1975). The value of the k_K factor for paddy field soil tended to be smaller than the values for upland field soils in the present study; the differences in the values between paddy field soil under a drained condition and upland field soils in CM of Togo and Ano were significant ($P < 0.05$, Tukey test). The factors for upland field soils showed greater values than those (0.18–0.24) for cornfield soils with various textures – fine sand, silt loam, and clay by Lorenz *et al*. (2010). The method for extraction of K from soil in the determination was almost same as that in the present study; i.e., Lorenz *et al*. (2010) added 50 mL of 1 M ammonium acetate to 5 g soil with shaking at 200 rpm for 20 min though the ratio of soil to extractant and the duration of shaking were different from those in the present study as described in Section 2. Therefore, these differences could be attributed from different compositions and populations of bacterial and fungal cultures selectively grown from the soils as mentioned by Lorenz *et al*. (2010), which may have influenced the extractability of K from the microbial cells. In addition, absorption of K to sites in minerals or organic matter in soil after chloroform-fumigation, from where K could not be extracted with 1 M ammonium acetate, might be a reason for the smaller values, in particular for paddy field soil. The average value of k_K factor is 0.37 for all data ($n = 8$) reported so far by Lorenz *et al*. (2010) ($n = 3$) and in the present study ($n = 5$) (Table 3); the value is 0.33 for paddy field soil ($n = 2$) and 0.42 for upland field soil ($n = 6$) though it would be still early to generalize these values for agricultural soils.

Table 3. Bacterial/fungal ratios (B:F), fractional conversion factors for bacteria (k_{Kb}) and fungi (k_{Kf}) and final conversion factor for microbial biomass potassium (k_K) after chloroform-fumigation extraction in paddy and upland field soils.

Soil	B:F[a]	k_{Kb}	k_{Kf}	k_K
Paddy field soil (Anjo; flooded)	35:65	0.46	0.34	0.38^{AB}
Paddy field soil (Anjo; drained)	35:65	0.37[b]	0.23[b]	0.28^{A}
Upland field soil (Togo; CF plot)	40:60	0.32	0.48	0.41^{AB}
Upland field soil (Togo; CM plot)	38:62	0.47	0.52	0.50^{B}
Upland field soil (Ano; greenhouse)	44:56	0.64	0.81	0.73^{C}

[a] Ratio of bacterial biomass to fungal biomass determined by the substrate-induced respiration method; [b] data from Yamashita *et al*. (2014).
CF, chemical fertilizer; CM, chemical fertilizer with farmyard manure.
Different letters (A, B, C) indicate a significant difference ($P < 0.05$, Tukey test).

Acknowledgments

We thank Professor Emeritus Makoto Kimura from the Graduate School of Bioagricultural Sciences, Nagoya University, for his helpful suggestions and comments. This work was supported in part by the JSPS KAKENHI Grant Number 15K07337.

Funding

This work was supported in part by the JSPS KAKENHI (Grant Number 15K07337).

References

Anderson JPE, Domsch KH 1973: Quantification of bacterial and fungal contributions to soil respiration. *Arch. Mikrobiol.* **93**, 113–127. doi:10.1007/BF00424942

Anderson JPE, Domsch KH 1975: Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. *Can. J. Microbiol.* **21**, 314–322. doi:10.1139/m75-045

Bailey VL, Bolton H Jr, Smith JL 2008: Substrate-induced respiration and selective inhibition as measures of microbial biomass in soils. *In* Soil Sampling and Methods of Analysis, 2nd ed., Eds. Carter MR, Gregorich EG, pp. 515–526.CRC Press, Boca Raton.

Castellano SD, Dick RP 1991: Modified calibration procedure for the measurement of microbial sulfur in soil. *Soil Sci. Soc. Am. J.* **55**, 283–285. doi:10.2136/sssaj1991.03615995005500010049x

Hammer H, Harper DAT, Ryan PD 2001: PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **4**, 9.

Helmke PA, Sparks DL 1996: Lithium, sodium, potassium, rubidium, and cesium. *In* Methods of Soil Analysis, Part 3. Chemical Properties, Ed. Sparks DL, pp. 561–562. SSSA, Madison.

Inubushi K, Wada H, Takai Y 1984: Determination of microbial biomass-nitrogen in submerged soil. *Soil Sci. Plant Nutr.* **30**, 455–459. doi:10.1080/00380768.1984.10434710

Islam TMD, Toyota K 2004: Susceptibility of soils with or without repeated application of farmyard manure to bacterial wilt of tomato caused by *Ralstonia solanacearum*. *Soil Microorg.* **58**, 33–42. doi:10.18946/jssm.58.1_33

Katayama A, Hu H-Y, Nozawa M, Yamakawa H, Fujie K 1998: Long-term changes in microbial community structure in soils subjected to different fertilizing practices revealed by quinone profile analysis. *Soil Sci. Plant Nutr.* **44**, 559–569. doi:10.1080/00380768.1998.10414479

Lorenz N, Verdell K, Ramsier C, Dick RP 2010: A rapid assay to estimate soil microbial biomass potassium in agricultural soils. *Soil Sci. Soc. Am. J.* **74**, 512–516. doi:10.2136/sssaj2008.0391

Mitsuboshi M, Kioka Y, Noguchi K, Asakawa S 2018: Evaluation of suppressiveness of the soils exhibiting soil-borne disease suppression after long-term application of organic amendments by the co-cultivation method of pathogenic *Fusarium oxysporum* and indigenous soil microorganisms. *Microbes Environ.* **33**, 58–65. doi:10.1264/jsme2.ME17072

Vance ED, Brookes PC, Jenkinson DS 1987: An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703–707. doi:10.1016/0038-0717(87)90052-6

Yamashita K, Honjo H, Nishida M, Kimura M, Asakawa S 2014: Estimation of microbial biomass potassium in paddy field soil. *Soil Sci. Plant Nutr.* **59**, 337–346. doi:10.1080/00380768.2014.919237 (Corrigendum [2016] **62**, 570–573. doi:10.1080/00380768.2016.1234137).